THE JOURNAL OF DAIRY RESEARCH

EDITED FOR

The Committee of Management

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VOLUME 12

CAMBRIDGE
AT THE UNIVERSITY PRESS
1941

PRINTED IN GREAT BRITAIN

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269. EXPERIMENTS ON THE CHEMICAL ENRICHMENT OF COWS' MILK BY THE ADMINISTRATION OF DIETHYLSTILBOESTROL AND ITS DIPROPIONATE

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(With 6 Figures)

IT was shown by Folley (1) that administration of oestrogens derived from natural sources (oestrone and oestradiol monobenzoate) will, under certain conditions, cause prolonged increases in the fat and non-fatty solids content of cows' milk. As is well known, oestrogen administration during lactation will often inhibit milk secretion (see Folley (2) for references), but in the above-mentioned experiments conditions were fortunately such that inhibition was incomplete and only temporary. This work indicated a possible method of experimentally increasing the solids content of cows' milk from the normal udder, in cases where it is desirable to do so, e.g. when the content of non-fatty solids is lower than the legal minimum of 8·5 %, but, unfortunately, practical application was precluded by the high price of naturally occurring oestrogens. The subsequent discovery by Dodds, Golberg, Lawson & Robinson (3) of the powerful oestrogenic activity of the relatively cheap synthetic stilbene derivative, diethylstilboestrol, opened up the possibility of utilizing Folley's method (1) on a practical scale and provided an incentive to undertake further necessary experimental work.

As a first step it was required to show that diethylstilboestrol, like oestrogens from natural sources, would increase the solids content of cows' milk, and a preliminary account of an experiment demonstrating that this was so was given by Folley & Scott Watson (4). The present paper describes further experiments designed to determine the conditions under which diethylstilboestrol will cause prolonged enrichment of cows' milk without at the same time decreasing the yield. The necessity of finding a practical method of administration and of obtaining information on the effect of diethylstilboestrol on the breeding capabilities of the cows were also kept in mind.

EXPERIMENTAL

Methods

The experimental animals were Ayrshire, British Friesian, Dairy Shorthorn and Guernsey cows belonging to herds under first-class management. Unless otherwise stated the cows were not pregnant at the time of experiment. The cows were milked twice daily and the yields recorded to the nearest $\frac{1}{4}$ lb. Milk

analysis was confined to determinations of fat content (Gerber) and non-fatty solids content (Golding (5)). In all experiments the administration of oestrogen was preceded by a preliminary control period during which observations on milk yield and composition were made.

Oral administration of diethylstilboestrol

Diethylstilboestrol exerts its characteristic oestrogenic action when given orally (Dodds *et al.* (3)), and since oral administration is the simplest method for practical use it was deemed necessary to study the effect on milk composition of diethylstilboestrol given by mouth.

During her third month of lactation, a Shorthorn cow, Cherry 22, was given 1 g. of diethylstilboestrol suspended in 400 ml. water in the form of a drench. Observations on milk yield and composition are plotted in Fig. 1. Since preliminary inspection of the results of this experiment suggested that the changes in fat content were such as might obscure any changes in the non-fatty solids percentages, the latter were calculated on the basis of fat-free milk "serum". It is seen that the oral administration of diethylstilboestrol was followed by relatively slight increases in fat and non-fatty solids percentages, though it should be noted that the highest level of non-fatty solids following the feeding of oestrogen was no higher than at the start of the control period. Milk yield suffered no appreciable diminution. It seemed probable that in order to effect appreciable and prolonged changes in the milk solids content by feeding diethylstilboestrol, regular feeding of relatively large quantities would be necessary. Since the practical possibilities of this form of administration seemed severely limited no further experiments on oral administration were made.

Injection of diethylstilboestrol dipropionate dissolved in oil

It is well known that esterification results in considerable prolongation of the activity of naturally occurring oestrogens, and Dodds *et al.* (6) showed that this also applied to diethylstilboestrol. Of a number of esters examined by them the dipropionate exhibited the most prolonged effect without undue loss of activity. Accordingly, since the object of these experiments was to produce *prolonged* changes in milk composition, the dipropionate was preferred to diethylstilboestrol itself for use in the following injection experiments, in which three Shorthorn cows were used.

Twenty-six days after the feeding experiment, Cherry 22 was given a series of five subcutaneous injections of 50 mg. diethylstilboestrol dipropionate in 2 ml. sesame oil (Fig. 1). These injections appeared to cause a slight rise in the milk non-fatty solids content, while the yield suffered a slight and temporary decrease.

A second cow, Flora 29, received a single subcutaneous injection of 400 mg. of the dipropionate in 5 ml. sesame oil in the eleventh month of lactation, when her daily milk yield was approximately 15 lb. The injection was followed by a sharp increase in milk fat and non-fatty solids percentages, but the yield

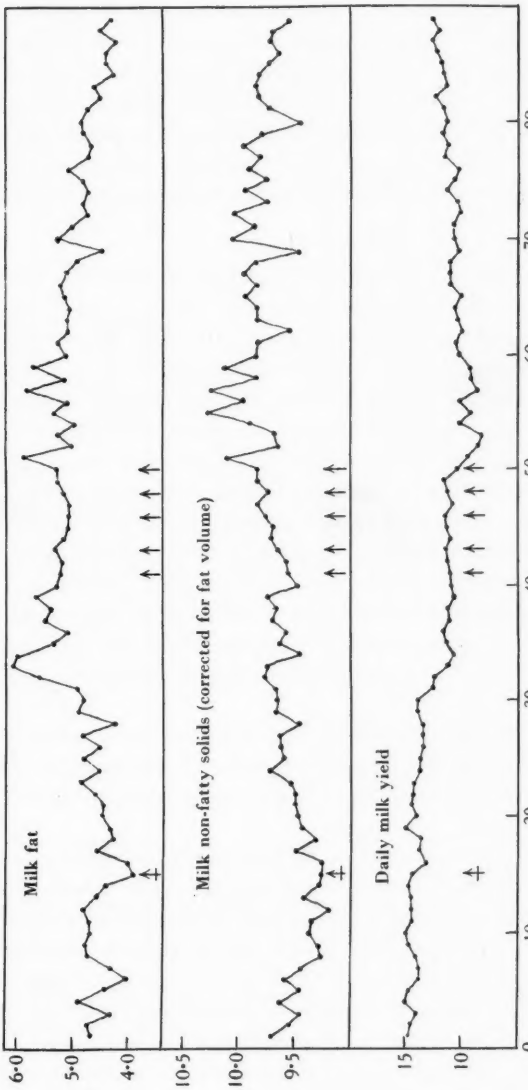

Fig. 1. Effect of feeding diethylstilboestrol and injection of its dipropionate on milk secretion in the Shorthorn cow, Cherry 22. At ↓, 1 g. diethylstilboestrol suspended in 400 ml. water was given by mouth during the third month of lactation. Each ↑ represents a subcutaneous injection of 50 mg. diethylstilboestrol dipropionate in 2 ml. sesame oil.

declined rapidly and the cow soon dried off despite attempts to prevent this by treatment with prolactin and then thyroxine.

A third cow, Fillpail 18, was given a single subcutaneous injection of 498 mg. of the ester dissolved in 5 ml. sesame oil. This cow had been in lactation 1 month at the time of injection and was giving approximately 27 lb. of milk daily. In this case the injection was followed by a rise in milk fat and non-fatty solids which had subsided, as nearly as can be estimated from the curves (not shown), by the end of 20 days. From the day of injection to the end of the observation period (35 days) there was a progressive decline in milk yield to a level of about 10 lb. daily.

Percutaneous inunction of the udder with diethylstilboestrol dipropionate

In many cases the growth response of an external organ such as the capon comb or the mammary gland can be elicited by a much smaller dose of the appropriate sex hormone, directly applied by inunction of an ointment or oily solution, than is necessary by systemic injection (see Deanesly & Parkes (7) for the capon comb and de Fremery (8) for the mammary gland). Thus Deanesly & Parkes (7) found that male hormone was some 200 times more effective in stimulating the growth of the capon comb when applied directly than when injected systemically. It seemed likely, therefore, that inunction of the udder might prove an economical way of administering diethylstilboestrol to cows for the purpose of influencing milk composition. Moreover, should the required changes in milk composition be brought about with relatively small doses of diethylstilboestrol by this method of administration, undesirable side effects might be less likely. Again, this method, if effective, would be quite easy to carry out in practice.

In all experiments involving inunction of the udder, control cows were treated with inactive ointment, in view of the possibility that the udder massage accompanying application of an ointment might itself influence milk yield or composition.

Two Shorthorn cows were used in the first experiment by inunction. The experimental cow, Flora 29, received eight applications at intervals of about 4 days of 5 g. of ointment containing 1 % diethylstilboestrol dipropionate. The ointment base consisted of lard and olive oil and each dose was gently applied to the skin of the udder and rubbed well in. At the time the treatment began she was in the ninth month of lactation and was giving about 30 lb. of milk daily. The control cow, Blossom, received similar treatment with inactive ointment base. The figures for the yield and composition of the milk of the experimental cow, which are given in Fig. 2, show that the treatment caused a striking rise in the milk non-fatty solids which appeared to begin after the third treatment. The milk-fat content also rose. It is interesting to note that immediately after the last treatment the milk solids began to fall and rapidly returned to the pre-treatment level. No diminution of milk yield occurred. The temporary nature of the response to the esterified oestrogen given by inunction in

this experiment stands in marked contrast to the prolonged response of the capon comb to testosterone propionate directly applied (Deanesly & Parkes(7)).

Two groups of four Friesian cows were available for a second inunction experiment. In both groups two cows were in mid-pregnancy when the inunctions began and two were believed to be in early pregnancy. A point of added interest regarding this experiment was that during the preliminary control period the mean non-fatty solids level for both groups of cows was

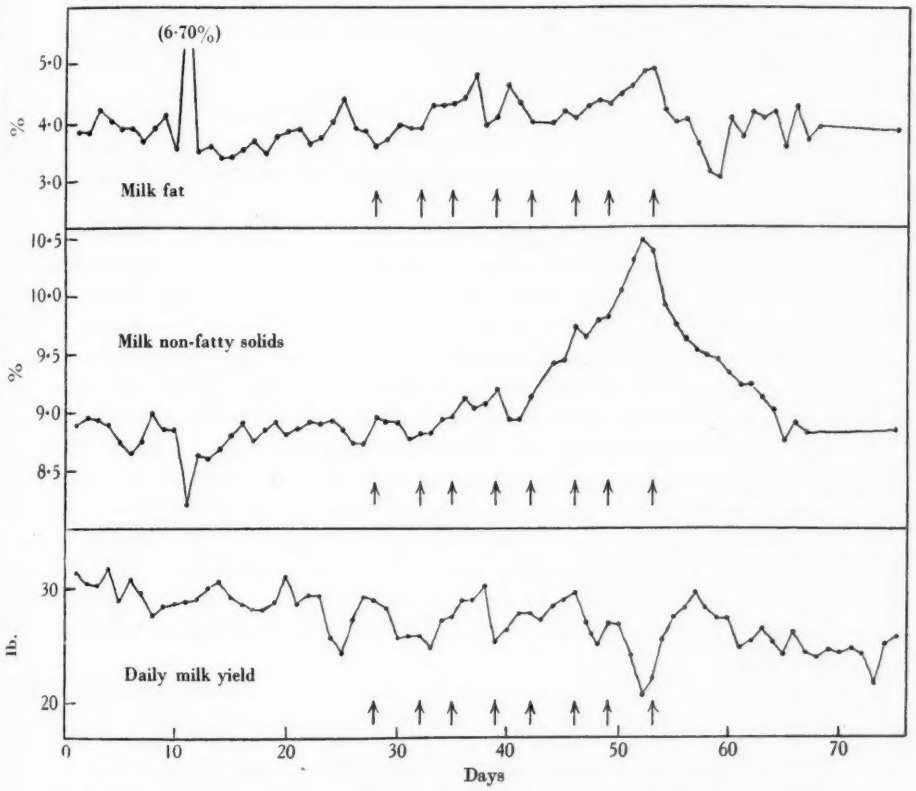

Fig. 2. Effect on milk secretion of percutaneous inunction of the udder of the Shorthorn cow, Flora 29, with diethylstilboestrol dipropionate. 5 g. ointment containing 1% diethylstilboestrol dipropionate were rubbed into udder at ↑. Total dose = 400 mg. diethylstilboestrol dipropionate. When the inunctions began Flora 29 was in the ninth month of lactation.

slightly below the legal minimum (8·5 %), so that it provided an opportunity of trying to improve the chemical quality of milk of poor quality from the legal point of view.

One group received applications of ointment containing stilboestrol dipropionate, while the other was similarly treated with ointment base. Five inunctions, each with 5 g. of 1 % ointment, were evenly spread over the first

15 days of the experiment; then, since no change in milk composition had occurred, six inunctions followed, each with 10 g. of the same ointment, spread over the next 14 days; finally, since the changes in milk composition at this stage were very slight, two inunctions each with 10 g. of 2 % ointment were given. The experiment was ended at this point because the two experimental cows in advanced pregnancy aborted (see Folley & Scott Watson(9)). Each cow thus received a total dose of 1·25 g. of ester, equivalent to 882 mg. of free diethylstilboestrol. In no case did the treatment cause any appreciable changes in milk yield or composition, though the average figures for the four experimental cows indicated a very slight rise in milk non-fatty solids towards the end of the inunction period.

It is noteworthy that in this experiment a high total dose of diethylstilboestrol, sufficient to induce abortion in two cows in advanced pregnancy, had no effect on milk secretion even though applied directly to the udder, though in the experiment with Flora 29 a total dose of 200 mg. of the dipropionate caused a marked though temporary increase in milk solids. In view of the possibility that breed differences were involved, a third inunction experiment was made on two Guernsey cows. One of these cows in the sixteenth month of lactation received eight inunctions of 2·5 g. of 1 % ointment spread over 21 days, followed by thirteen inunctions of 5 g. of the same ointment spread over 41 days, a total of 850 mg. of the dipropionate being applied. The other cow was given similar treatment with ointment base. There was no change in milk yield or composition during the period of treatment with the lower dose. At about the time of the fifth treatment with the higher dose the non-fatty solids content rose from a mean level of about 9 % to about 9·3 % and persisted at this level until the end of the observation period, 19 days after the last inunction. There was a fall in the mean milk yield level from about 19 lb. daily to a minimum of about 15 lb. daily during the first part of the period of treatment with the higher dose, but thereafter the yield returned to a mean level of about 18 lb. daily.

In all the inunction experiments observations on the control cows showed that inunction with ointment base alone had no effect on lactation.

Subcutaneous implantation of crystalline diethylstilboestrol

It was shown by Deanesly & Parkes(7) that subcutaneous injection of partly crystallized oily solutions of sex hormones and, in particular, subcutaneous implantation of solid tablets, resulted in great prolongation of action. This method of administration of diethylstilboestrol was therefore tried on a Shorthorn cow, Blossom, in the eighth month of lactation. A small incision was made in the skin of the neck under Percaine local anaesthesia and a small space between the skin and the underlying tissues opened by blunt dissection. Into this space was poured approximately 900 mg. of powdered diethylstilboestrol. The incision was closed by two stitches. No local reaction was subsequently noticed. The treatment caused a striking increase in the milk

solids, which lasted as long as observations were continued (96 days). For some 34 days after the implantation the milk yield remained at the pre-injection level; it then slowly fell, but this was probably due to the normal decline in lactation rather than to the effect of the treatment (Fig. 3). This experiment may be considered a spectacular success as regards the practical objective of this work, but suffers from the drawback that the method of administration used is hardly one that would be useful in ordinary practice.

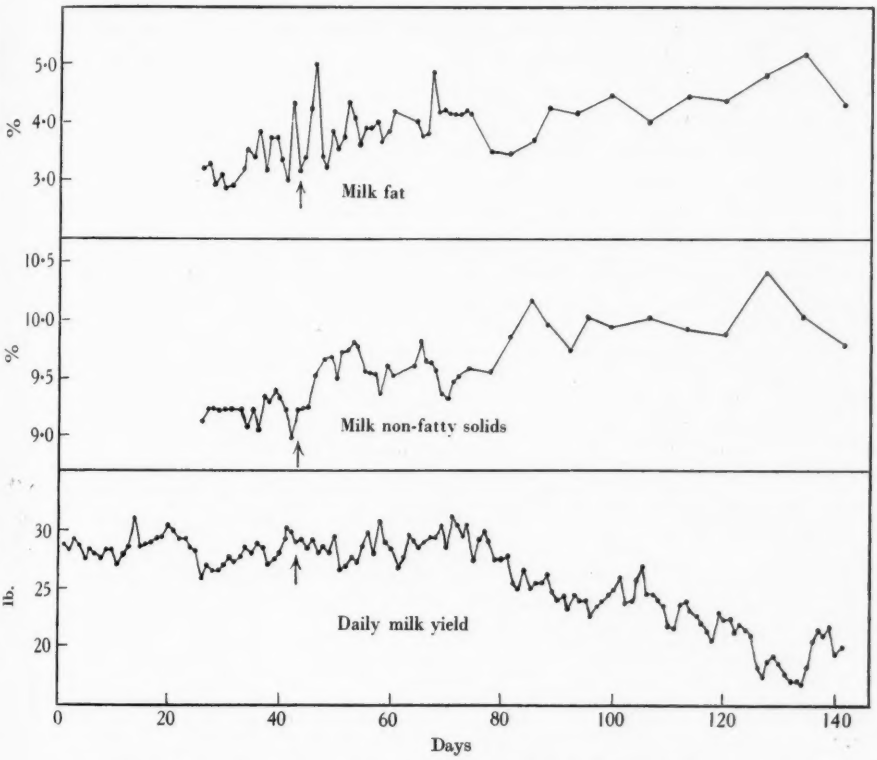

Fig. 3. Effect of subcutaneous implantation of crystalline diethylstilboestrol on milk secretion in the Shorthorn cow, Blossom. Approximately 900 mg. diethylstilboestrol crystals were subcutaneously implanted at ↑. At the time of treatment Blossom was in the eighth month of lactation.

Subcutaneous injection of an aqueous suspension

Since diethylstilboestrol is relatively insoluble in water, it occurred to us that the same result could probably be more simply attained by the subcutaneous injection of an aqueous suspension. Accordingly, the same cow, Blossom, in the second month of the next lactation, was given a subcutaneous injection of 1 g. diethylstilboestrol suspended in 80 ml. water. The diethylstilboestrol was thoroughly ground up with the water and the mixture well shaken before taking

up into the syringe. In passing it may be remarked that in making injections of this type, trouble is sometimes experienced owing to blocking of the needle unless the latter is of fairly large bore. Again a very successful result was obtained. The milk solids showed marked increases which lasted for some 180 days: the milk yield was unaffected by the treatment (Fig. 4).

This experiment was repeated on three Ayrshire cows, two of which at the time of injection were in the second month of lactation while the other was at the end of the first month. Each cow received 1 g. of diethylstilboestrol. In all cases the injection was followed by striking increases in the concentrations of the milk solids accompanied, unfortunately, by a progressive decline in milk yield. The milk yield and solids content for one of these cows, Cinderella, are shown in Fig. 5. These curves are typical of all three experiments. The decline in milk yield may have been partly due to lack of grass associated with a period of prolonged drought which set in about the time of the injections, but milk yield figures, during the same period, of cows which had calved on about the same dates as the experimental ones indicate that this would not wholly account for the decline. One is therefore forced to conclude that in these three cases the treatment had caused an appreciable decrease in milk yield.

A further experiment was made on two Shorthorn cows which were in the second month of lactation, in order to determine whether smaller doses of diethylstilboestrol would raise the milk solids without adversely affecting the yield. The first, Fillpail 18, was given 375 mg. of diethylstilboestrol suspended in 22 ml. water, while the other, Cherry 15, received 225 mg. With the larger dose the injection was followed by relatively slight but definite increases in milk solids, which persisted for about 20 days (Fig. 6). There was a decrease of about 10 lb. in the daily milk yield, but again it is possible that this was largely due to adverse weather conditions, since the injection coincided with the beginning of an exceptionally severe and prolonged spell of cold weather. No changes in milk composition were observed in the cow, Cherry 15.

Effect of diethylstilboestrol on pregnant cows

In a previous section it was mentioned that application, by inunction of the udder, of a total of 1·25 g. diethylstilboestrol dipropionate produced abortion in two British Friesian cows in advanced pregnancy. A fuller account of this aspect of the experiment was published elsewhere (Folley & Scott Watson (9)). In view of the importance of this phenomenon from the point of view of the use of diethylstilboestrol in practice, the effect of administration of this apparently very potent abortifacient was further studied on a group of three pregnant Ayrshire cows. Each cow was given a subcutaneous injection of 1 g. diethylstilboestrol dipropionate suspended in 22 ml. water. Two of the cows aborted within 5 weeks of the injection: in the other the effect was delayed, an 8 months' calf being prematurely delivered 2 months after the injections. The relevant data are given in Table 1.

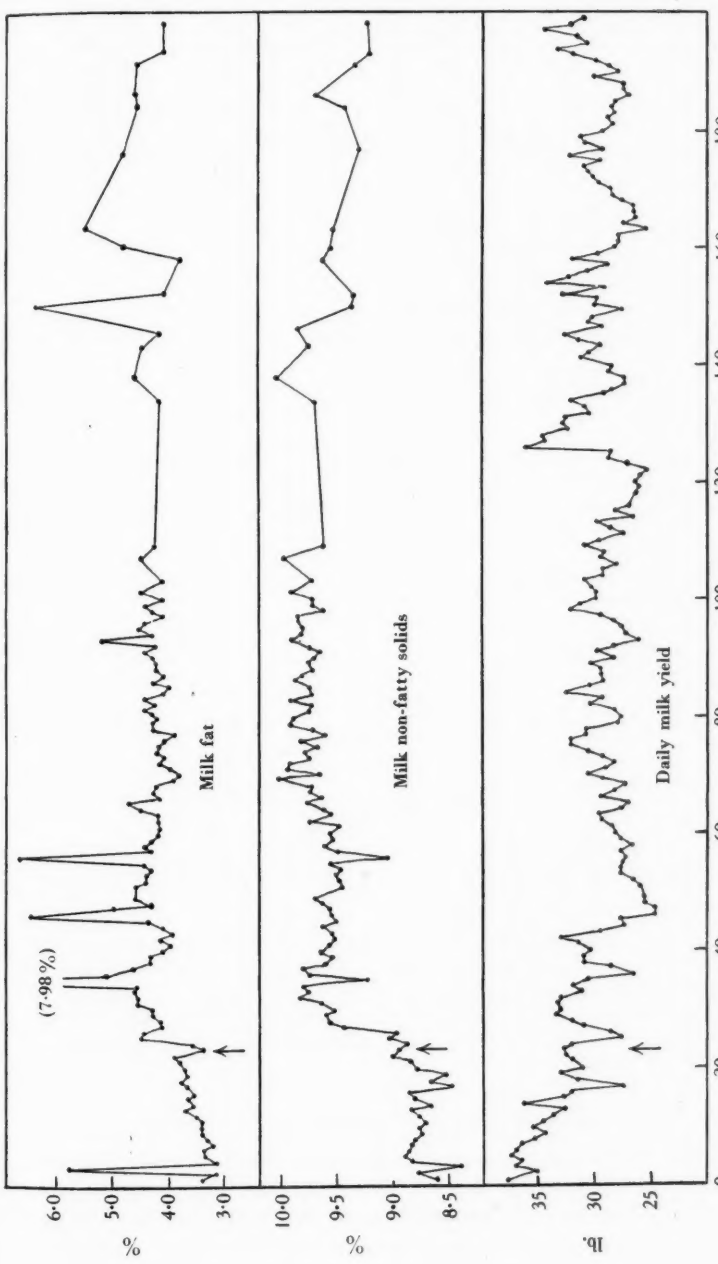

Fig. 4. Effect of subcutaneous injection of an aqueous suspension of diethylstilboestrol on milk secretion in the Shorthorn cow, Blossom. A suspension of 1 g. diethylstilboestrol in 80 ml. water was subcutaneously injected at ↑. At the time of injection Blossom was in the second month of lactation.

Table 1. *Effect of diethylstilboestrol on pregnancy in the cow*

Cow	Days pregnant at the date of injection	Date of injection	Aborted	Remarks
Gitta	184	11. vi. 40	7. vii. 40	Dead foetus
Favourite Beauty	191	11. vi. 40	17. vi. 40	Dead foetus
Primula	193	11. vi. 40	4. viii. 40	Live calf

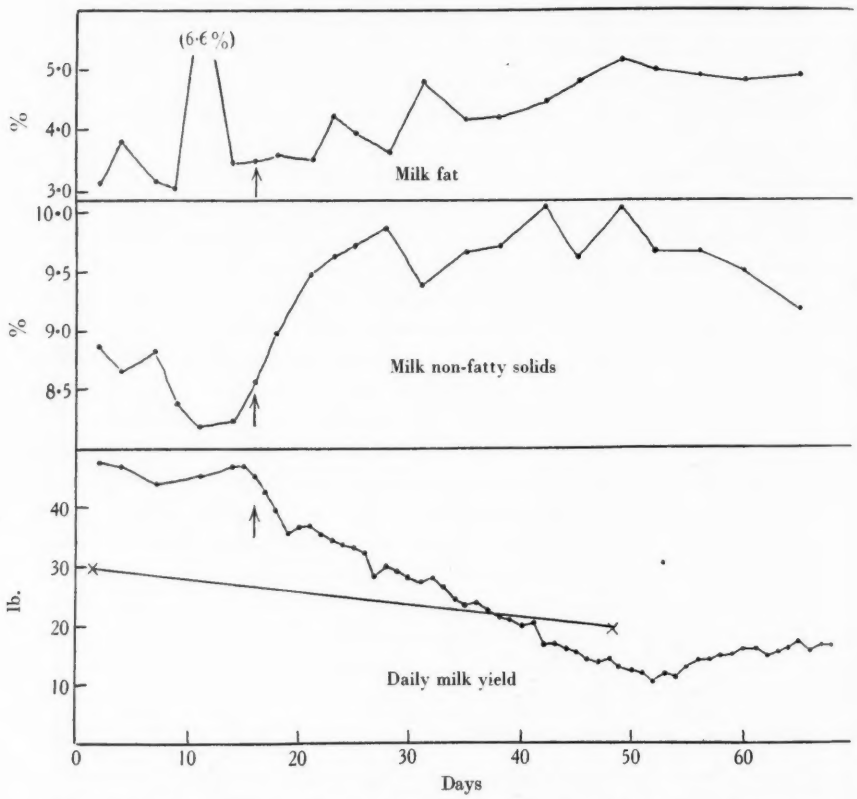

Fig. 5. Effect of subcutaneous injection of an aqueous suspension of diethylstilboestrol on milk secretion in the Ayrshire cow, Cinderella. A subcutaneous injection of 1 g. diethylstilboestrol suspended in 50 ml. distilled water was given at ↑. At the time of injection Cinderella was in the first month of lactation. × × represent mean daily milk yields of the Ayrshire cow, Star 2, which calved 2 days before Cinderella and was kept under the same conditions during the experimental period.

Effect of diethylstilboestrol on reproduction in the cow

Another consideration of importance in connexion with the possible practical application of diethylstilboestrol treatment in the cow is the effect of the treatment on the subsequent breeding history. The data regarding the subsequent breeding performances of those of the cows used in these experiments about which sufficient was known at the time of writing are given in Table 2.

Table 2. *Reproductive histories of cows treated with diethylstilboestrol and its dipropionate*

Cow	Total dose mg.	Route of administration	Date of treatment	Remarks
		(*a*) Cows treated with diethylstilboestrol		
Blossom	900	Subcutaneous implantation	12. vii. 38	Calved on 24. vii. 39, requiring one service
Blossom	1000	Subcutaneous injection of aqueous suspension	26. ix. 39	Has had seven services since treatment, but may now be in calf. Slight symptoms of nymphomania
Cherry 15	225	Subcutaneous injection of aqueous suspension	5. ii. 40	Served on 3. v. 40 and has calved to that service
Fillpail 18	375	Subcutaneous injection of aqueous suspension	5. ii. 40	Served on 11. ii. 40. 13. ii. 40 and 27. ii. 40, but now believed barren. Exhibits symptoms of nymphomania
Cherry 22	1000	Oral	11. xi. 38	See below
		(*b*) Cows treated with diethylstilboestrol dipropionate		
Cherry 22	250	Subcutaneous injection of oily solution	7–16. xii. 38	Served on 19. x. 38, 10. i. 39 and 1. iv. 39. Since sold barren. She had suffered from reproductive troubles before the experiment
Flora 29	400	Subcutaneous injection of oily solution	2. ii. 39	Calved on 9. v. 40, requiring eight services after treatment (one before)
Flora 29	400	Inunction	6–31. x. 38	See above
British Friesian: Cow No. 1	1250	Inunction	16. v. 39–19. vi. 39	Aborted on 21. vi. 39 and since calved on 9. vi. 40, requiring three services
Cow No. 2	1250	Inunction	16. v. 39–19. vi. 39	Aborted on 21. vi. 39 and since calved on 29. ix. 40, requiring seven services
Cow No. 3	1250	Inunction	16. v. 39–19. vi. 39	Served on 12. iii. 39, 5. viii. 39 and calved on 12. v. 40
Cow No. 4	1250	Inunction	16. v. 39–19. vi. 39	Calved on 2. ii. 40 to a service on 28. iv. 39. Served on 11. vi. 40, 21. vii. 40, 11. viii. 40 and now known to be in calf. Had exhibited ovarian hypo-activity before the treatment

In addition to the information given in Table 2 it should be stated that two of the three non-pregnant Ayrshire cows, which received subcutaneous injections of 1 g. diethylstilboestrol in aqueous suspension exhibited, some time after the injections, typical symptoms of nymphomania, namely, raised tail-head, sunken pelvic bones, and a persistent or frequently recurring tendency to ride, or be ridden by, other cows. Palpation of the ovaries of these two cows *per rectum* revealed no evidence of the presence of ovarian cysts or indeed of any abnormality whatever.

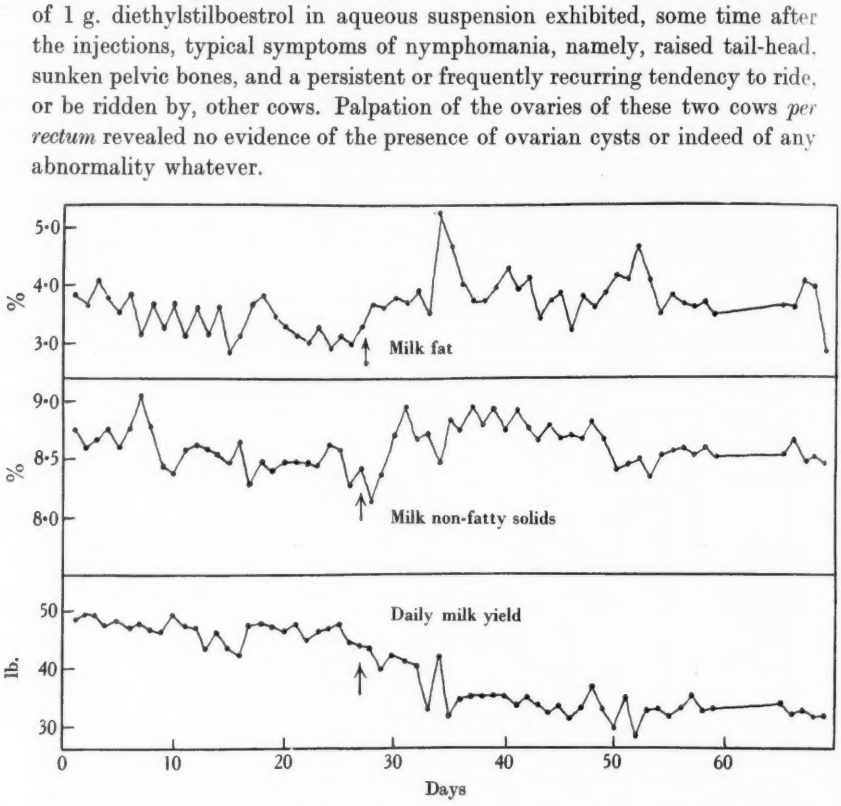

Fig. 6. Effect of subcutaneous injection of an aqueous suspension of diethylstilboestrol on milk secretion in the Shorthorn cow, Fillpail 18. A subcutaneous injection of 375 mg. diethylstilboestrol suspended in 22 ml. distilled water was given at ↑. At the time of injection Fillpail 18 was in the second month of lactation.

Discussion

A noteworthy feature of these experiments is the variability of the results. What may be called the positive ones, i.e. experiments in which a significant, if not always prolonged, increase in milk solids content occurred, can be divided into two classes, those in which no apparent decline in milk yield occurred and those in which the treatment was followed by an obvious fall in milk yield.

A closer analysis (see Table 3) of the results of the three experiments belonging to the first class, in which marked increases in milk solids content were observed, shows that while in two of them the treatment was followed by a slight (about 6 %) reduction in the yield of fat-free milk "serum", there was no inhibition of lactation in a real sense, because in all three experiments the total

amount of fat and non-fatty solids secreted over 14 days after the treatment was greater than over the corresponding pre-treatment period. This in itself indicates that the mechanism of the improvement in milk quality brought about by oestrogen administration is not a mere concentration of milk solids due to secretion of less water by the mammary gland, a conclusion which is borne out by the figures given in the last three lines of Table 3. In favourable cases, therefore, oestrogen administration seems to cause a real increase in the amount of milk solids secreted by the mammary gland, and in this connexion it is interesting to recall that Folley, Scott Watson & Bottomley (10) found that inunction of the udder region in virgin goats with suitable doses of diethylstilboestrol dipropionate induced copious lactation.

Table 3. *Effect of diethylstilboestrol on the composition of cows' milk*

	(1) Blossom (implantation of crystalline diethylstilboestrol)	(2) Blossom (injection of aqueous suspension of diethylstilboestrol)	(3) Flora 29 (inunction of udder with diethylstilboestrol dipropionate)
Total fat secreted (lb.)			
(*a*) During 14 days prior to treatment	13·40	16·50	14·92
(*b*) During 14 days after treatment	15·32	20·12	16·06
Total non-fatty solids secreted (lb.)			
(*a*) During 14 days prior to treatment	36·31	40·12	35·10
(*b*) During 14 days after treatment	38·13	41·16	35·65
Total fat-free milk "serum" secreted (lb.)			
(*a*) During 14 days prior to treatment	381·4	441·0	382·1
(*b*) During 14 days after treatment	383·2	413·6	357·7
Mean fat %			
(*a*) Over 14 days prior to treatment	3·39	3·61	3·76
(*b*) Over 14 days after treatment	3·84	4·64	4·30
Mean non-fatty solids % (in fat-free "serum")			
(*a*) Over 14 days prior to treatment	9·52	9·10	9·19
(*b*) Over 14 days after treatment	9·94	9·95	9·97
Change in mean fat % expressed as % of mean level before treatment	+13·2	+28·6	+14·3
Change in mean non-fatty solids % expressed as % of mean level before treatment	+ 4·5	+ 9·4	+ 8·5
Change in yield of fat-free milk "serum" expressed as % of mean level before treatment	+ 0·7	− 6·2	− 6·4

In the three experiments in question the increase in milk-fat secretion was much greater than the increase in the non-fatty solids. It may be pointed out that this last feature was also found to be characteristic of the effects of thyroxine on milk secretion (Folley & White (11)), though at first sight it would hardly seem probable that these changes in milk composition are mediated by the thyroid gland, since there is evidence that oestrogen administration causes hypo-activity of the thyroid (Bacsich & Folley (12)). It may be, of course, that low levels of oestrogen, levels which are insufficient to cause inhibition of lactation, may activate rather than depress the activity of the thyroid (see below).

These considerations indicate that the effects on lactation which are exerted by diethylstilboestrol, and indeed by oestrogens in general, namely inhibition on

the one hand and the increase in the milk solids content (enrichment effect) on the other, are partially independent, since in these experiments inhibition has always been accompanied by enrichment, while the converse has not always been true. It may be supposed, therefore, that the threshold for enrichment is lower than for inhibition. In the light of modern knowledge of the key role played by the anterior pituitary in the control of lactation (see Folley (2)), it seems likely that the anterior pituitary is concerned in both effects, low levels of oestrogen stimulating the anterior lobe to increased production of certain hormones connected with lactation, e.g. thyrotrophin, while higher levels, as is well known, suppress the secretion of many anterior lobe hormones. Evidence bearing on this point is discussed by Severinghaus (13). It may be noted that if this theory were true, one would expect that udder inunction would be a no more, indeed probably a less efficient method of administration than systemic injection; on the other hand, if enrichment is due to a direct action of oestrogen on the mammary gland, local inunction should be the most economical technique. The present data do not allow of a decision between these two theories.

In order to make practical use of the "enrichment effect" of oestrogens it is necessary to find conditions of administration under which only the lower threshold is exceeded in the bovine circulation. In this respect the present experiments have given less information than was hoped for. Oral administration, the simplest method for field use, proved disappointing, while another convenient technique, namely inunction of the udder, gave variable results in three experiments, which might possibly have been due to breed differences in the rate of absorption of the diethylstilboestrol dipropionate from the site of application and its subsequent metabolic disposal. The available data are not, however, sufficiently extensive to decide this. In two of three experiments involving subcutaneous injection of oily solutions, inhibition of lactation occurred, while the changes in milk composition were neither great nor very prolonged.

The most successful and indeed spectacular results were obtained by subcutaneous implantation of solid diethylstilboestrol and by the more convenient method of subcutaneous injection of an aqueous suspension. However, these two successes were only obtained on one particular cow, and when the method was repeated on other animals considerable inhibition of lactation occurred. This method of administration evidently possesses practical possibilities, but further work is necessary to determine the conditions under which no decline in milk yield may be expected to occur.

The experiments with pregnant cows clearly show that any method of improving the chemical quality of cows' milk involving the administration of large doses of oestrogen could not be applied to such cows, owing to the probability that abortion will result from the treatment. This would not seriously affect the practical value of such a treatment, should it ever become feasible, because the treatment would be of most value if given early in lactation, i.e. before the first service after calving. In passing it is interesting to note

that there are grounds for the supposition that diethylstilboestrol does not always interrupt pregnancy in the cow in its *earlier* stages. Of the two cows in early pregnancy reported upon by Folley & Scott Watson (9) there is now reason to believe that one may not have been pregnant at the time of the experiment, since, though she came into oestrus some time after the inunctions, no evidence of abortion was observed by a very competent herdsman. In the other, pregnancy continued and parturition was normal.

The available data on the effect of diethylstilboestrol on the subsequent reproductive performance of its recipients is not sufficiently extensive to enable hard and fast conclusions to be drawn. One cow, Blossom, received 1 g. of the substance during each of two successive lactations. After the first administration she readily became pregnant, but some difficulty has been experienced in stocking her after the second treatment. Similarly, the cow Flora 29, which was given diethylstilboestrol on two occasions during one lactation, has also been difficult to get in calf since. On the other hand, of the four British Friesians which received high does of this substance, three subsequently bred without difficulty. The position may be summed up by saying that in some cases cows which have received diethylstilboestrol treatment have subsequently proved difficult to get in calf, but the data as a whole provide no evidence that this is invariably so.

The incidence of nymphomaniac symptoms following the treatment in some cases is disquieting, and until conditions of administration which, while yielding the desired results as regards changes in milk composition, result in no unfavourable effects on reproductive ability and on milk yield are discovered, the administration of doses of the order of 500–1000 mg. diethylstilboestrol to cows cannot be considered as safe.

In previous experiments (Folley (1)) with oestrogens derived from natural sources (oestrone and oestradiol monobenzoate) no difficulty was experienced in eliciting the enrichment effect accompanied by only a transient and partial inhibition, whereas in the present experiments the two effects could often not be separated. This difference between the two sets of results may possibly be due to relatively slow inactivation of diethylstilboestrol in the organism after absorption from the site of injection, by contrast with the rapid inactivation of oestrogens from natural sources (see Zondek & Sulman (14)). This may also explain the occurrence of nymphomania in some cows after treatment with diethylstilboestrol, contrasted with the absence of breeding troubles after the administration of derivatives of naturally occurring oestrogens. Should this explanation prove to be correct it would indicate that naturally occurring oestrogens are likely to be more useful than diethylstilboestrol for the purpose discussed in this paper.

Another consideration of practical importance in connexion with these experiments may be referred to in conclusion. This concerns the possibility that cows which have been given diethylstilboestrol might excrete it in their milk. In view of the considerable oestrogenic activity of diethylstilboestrol by

the oral route and the consequent possibility of the occurrence of undesirable effects in consumers of the milk from treated cows, it is desirable to know whether or not, and if so in what amount, such milk contains diethylstilboestrol. A study of this aspect of the work is at present in progress.

Summary

1. Diethylstilboestrol administered orally to a Shorthorn cow had no marked effect on milk yield or composition.

2. A series of injections of the dipropionate in oily solution led to a slight rise in non-fatty solids in the same cow as in 1. Single larger injections were followed by a rise in milk solids accompanied by a rapid fall in milk yield in two other Shorthorns.

3. Inunction with an ointment containing the dipropionate led to a marked increase in milk solids in a Shorthorn cow, with no change in milk yield. The effect subsided rapidly when treatment was stopped. No significant effects were produced by similar treatment of four pregnant British Friesians; on increasing the dose two of these aborted. A Guernsey cow showed a slight increase in non-fatty solids and a slight but temporary fall in milk yield.

4. Subcutaneous implantation of crystalline diethylstilboestrol led to a striking and prolonged increase in milk solids, with no fall in milk yield, in a Shorthorn cow.

5. Subcutaneous injection of an aqueous suspension of diethylstilboestrol (1 g.) was equally successful when applied to the same cow as 4, but in the next lactation. In three Ayrshires the increase in solids was accompanied by an appreciable decline in milk yield. A Shorthorn receiving 375 mg. showed a temporary rise in solids, while one receiving 225 mg. showed no effect.

6. In all cases where milk yields declined the milk solids percentage rose, but the converse did not hold. Hence the threshold dose for inhibition is apparently higher than for enrichment.

7. The threshold doses may depend on the breed; the most successful results were obtained with Shorthorns.

8. Treated cows may be difficult to get in calf subsequently, especially those treated twice.

9. Administration of large doses of diethylstilboestrol to cows in advanced pregnancy results in abortion.

10. The enrichment of the milk in favourable cases represented a true increase in the yield of solids secreted, and not merely a concentration due to reduced secretion of water.

For the provision of experimental animals and willing co-operation in many ways we are indebted to Prof. R. Rae and Mr K. W. D. Campbell of the University of Reading Farm, Sonning, to Major G. W. Dunkin, Director, and Mr S. J. Edwards and Mr E. A. Macmillan of the Agricultural Research Council's

Field Station, Compton, and to our colleague, Mr James Mackintosh. Our thanks are also due to Mr A. Wagstaff of the Chemical Department of this Institute for determinations of milk solids. The diethylstilboestrol and its dipropionate were generously supplied by Dr F. L. Pyman, F.R.S. and Dr W. F. Short of Messrs Boots Pure Drug Co., Ltd., Nottingham. We would also express our gratitude to the Milk Marketing Board for a grant in aid of this work, which was carried out during the tenure by one of us (H. M. S. W.) of a Research Grant from the Agricultural Research Council.

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(*MS. received for publication* 12 *October* 1940)

576.809.31:576.85

270. LANCEFIELD GROUP B STREPTOCOCCI (*STR. AGALACTIAE*) ON THE HANDS OF MILKERS AND OTHERS

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The role of hands in the transference of pathogenic organisms is well appreciated. Various workers, notably Colebrook & Maxted (1), have in recent years studied the flora of hands and have suggested methods of sterilization after contact with infected material.

It is clear from references cited by Munch-Petersen (2), in his bibliography on mastitis, that it is well recognized that the streptococci causing contagious mastitis (*Str. agalactiae*) may be passed from cow to cow on the hands of milkers. The advice to wash the hands before and between the milking of cows and, more recently, to disinfect them with substances such as hypochlorites is frequently given, but no precise work upon the efficiency of the suggested procedures appears to have been carried out.

Attempts have been made to assess the bacterial flora of the skin of the hand and the relative efficiency of methods of sterilization. Colebrook & Maxted (1) have shown that treatment with various germicides after washing with soap and water will virtually eradicate haemolytic streptococci artificially implanted on the skin, but that the normal hand flora, staphylococci and sarcina were scarcely affected. They studied the skin flora by washing the skin surface with a sterile swab and made cultures from the swab. Other methods of studying the skin flora have been devised, such as scraping a given area and making impressions on prepared agar plates. All these results suggest that there is an established skin flora, usually of a saprophytic character and seldom containing pathogenic organisms unless contact with infected material has recently taken place. Price (3), however, claims that the skin flora is of two types: "resident" and "transient". The "resident" flora inhabits the skin and is virtually impossible to eradicate by ordinary means. The "transient" flora is acquired on contact with infected material and is easier to deal with. Price suggests, however, that frequent contact with the same type of infection may result in organisms, ordinarily "transient", becoming "resident". He showed further that successive scrubbings in water decreased the numbers of the "resident" flora logarithmically, and that the type of soap and the temperature of the water were less important factors than the mechanical cleansing action of scrubbing.

It may, therefore, well be that milkers frequently in contact with cows

suffering from *Str. agalactiae* infections may acquire a "resident" flora containing this organism which may be difficult to remove from the hands between the milking of successive cows. With this possibility in view the following investigation was carried out.

TECHNIQUE

In this series of experiments the hands were washed in enamel basins, previously sterilized by autoclaving, and containing about 500 ml. of sterilized milk. The subject of the experiment used a sterilized hard nail brush. The term "hands" included the palms, fingers and thumbs, the backs of the hands and the wrists. Special attention was paid to the nails. Each hand was scrubbed for 1½ min. One portion of 10 ml. of the milk was then centrifuged at 2500 r.p.m. for 10 min. and the deposit taken up in 2 ml. sterile saline; 0·5 ml. of this suspension was then plated in Edwards's (4) medium and incubated overnight at 37° C. The plates were then examined and suspicious colonies removed for identification. A further portion of 50 ml. was incubated overnight (37° C.) in a flask containing crystal violet, sufficient to give a final concentration of 1 in 500,000. If there were no likely colonies on the first plate the milk which had been incubated with crystal violet was streaked on Edwards's medium, incubated and examined. If no suspicious colonies were found on the plates from the centrifuged deposit it was assumed that the organism could only have been present in very small numbers—less than 1 in 2·5 ml.

IDENTIFICATION OF ORGANISMS ISOLATED

All suspicious organisms isolated were examined and their biochemical reactions and other characteristics determined. Those conforming to the following were assumed to be *Str. agalactiae*:

Morphology. Cocci, forming chains of varying length in dextrose-lemco broth.

Fermentation reactions. Acid, no gas in dextrose, sucrose, lactose, maltose and salicin. No visible reaction in mannitol, sorbitol, raffinose, inulin and aesculin. Sodium hippurate hydrolysed.

Reactions in milk. Methylene blue milk (5) 1 in 15,000. Not reduced.

Yeast-dextrose-litmus milk at 37° C. Acid clot, faint reduction with non-haemolytic and acid without clot with haemolytic strains.

Yeast-dextrose-litmus milk at 45° C. No growth.

Haemolysis. Both haemolytic and non-haemolytic types were isolated from Edwards's medium.

Precipitin reactions. HCl extracts of representative organisms from each washing were tested against a precipitating serum for Lancefield Group B streptococci (Lancefield technique).

EXPERIMENTAL

A routine method of disinfecting the milkers' hands during milking had been recommended for use on all the farms dealt with. The milker first washed his hands thoroughly with soap and water before milking and then rinsed his

hands in a solution of sodium hypochlorite containing about 800 p.p.m. available chlorine for 10–15 sec., after milking each cow. It was observed that under practical conditions both the strength of the solution and the time of rinsing varied considerably. Where the sodium hypochlorite solution was not used in the normal cowshed routine it was introduced for the purpose of the experiment.

Farm 1

This herd, of about forty cows, contained eight animals known to be suffering from streptococcus mastitis or harbouring the organism (6). Each milker on this farm dealt with from five to eight cows. The routine hand disinfection outlined above was observed, and in addition the hands were washed with soap and water after rinsing in the hypochlorite solution after milking the last cow. The results of the test scrubbings are given in Table 1.

Table 1

Milker	Test scrubbing	Treatment of hands before test scrubbing	Result: Lancefield Group B streptococci isolated from	
			Centrifuged deposit	Milk incubated with crystal violet
A	First	Milking completed. Routine disinfection as for Farm 1 above	Negative	Haemolytic type
	Second	Before p.m. milking. Soap and water wash only	Haemolytic and non-haemolytic types	Not tested
	Third	Milking completed. Routine disinfection as for Farm 1 above	Negative	Haemolytic type
	Fourth	Milking completed. Routine disinfection as for Farm 1 above	Negative	Haemolytic and non-haemolytic types
B	First	Before p.m. milking. Soap and water wash only	Non-haemolytic type	Not tested
	Second	Milking completed. Routine disinfection as for Farm 1 above	Haemolytic and non-haemolytic types	Not tested
	Third	Milking discontinued 10 days. Hands not washed	Haemolytic type	Not tested

Milker A was normally employed, while milker B was only temporarily occupied, in milking. He had been milking for about 14 days before the first test scrubbing and had stopped milking again some 10 days before the third. The second test scrubbing for milker A and the first for milker B took place in the afternoon about 8 hours after the previous morning's milking.

Farm 2

This farm was in a different locality from Farm 1. There were twenty-five to thirty cows, four of which showed clinical symptoms of mastitis. The remainder were known to be harbouring the organism as shown by Edwards's technique (6). Each milker on this farm dealt with from four to eight cows. Routine hand disinfection was observed. The results are set out in Table 2.

Table 2

Milker	Test scrubbing	Treatment of hands before test scrubbing	Result: Lancefield Group B streptococci isolated from: Centrifuged deposit	Milk incubated with crystal violet
C	First	Milking completed. Soap and water only, after milking each cow	Haemolytic and non-haemolytic types	Not tested
	Second	Milking completed. Routine disinfection	Haemolytic and non-haemolytic types	Not tested
D	First	Milking completed. Soap and water only, after milking each cow	Haemolytic and non-haemolytic types	Not tested
	Second	Milking completed. Routine disinfection	Negative	Haemolytic and non-haemolytic types
E	First	Milking completed. Routine disinfection	Negative	Non-haemolytic type

Farm 3

All the cows had been examined at monthly intervals for 8 months, using Edwards's technique, and any cow showing *Str. agalactiae*, however few in numbers, was removed to another farm. There were no positive results with this technique for some months previous to or following the period during which experimental hand washing took place (6). Using an enrichment technique, however (7), two positives had occurred. These animals were not removed from the farm. A milking machine was in use and stripping was done by hand. The numbers of cows on this farm varied from fifty-five to seventy and each milker handled twenty to twenty-five cows. The results of test scrubbings are shown in Table 3.

Table 3

Milker	Test scrubbing	Treatment of hands before test scrubbing	Result: Lancefield Group B streptococci isolated from: Centrifuged deposit	Milk incubated with crystal violet
F	First	Stripping completed. Routine disinfection	Negative	Negative
	Second	Stripping completed. Routine disinfection	Negative	Haemolytic type
G	First	Stripping completed. Routine disinfection	Haemolytic and non-haemolytic types	Not tested
	Second	Stripping completed. Routine disinfection	Negative	Haemolytic type
	Third	Stripping completed. Routine disinfection	Negative	Haemolytic type
H	First	Stripping completed. Routine disinfection	Haemolytic and non-haemolytic types	Not tested

The first test scrubbing for milker F was the only one throughout the experiment where no Lancefield Group B streptococci were isolated either from the centrifuged deposit or the milk incubated with crystal violet.

It is clear from the results detailed above that streptococci of Lancefield Group B are commonly to be found on the hands of milkers. Some of these milkers were working with a herd (Farm 3) which would be considered free from the disease by the usual routine method of examination (Edwards's technique). Actually two cows had been shown to be harbouring *Str. agalactiae* when using an enrichment method.

The regularity with which these organisms could be recovered from milkers' hands, even though the numbers were apparently small, suggested that they might have become part of the "resident" flora of the hands. That the streptococci isolated were *Str. agalactiae* of bovine origin is probable from the fact that all the milkers milked cows known to be harbouring the organism, and that the organisms gave the precipitin reactions of Lancefield Group B and the biochemical reactions of *Str. agalactiae*. Since, however, Hare & Colebrook(8) recovered Lancefield Group B streptococci from the human vagina and believed them to be of bovine origin, it was possible that other parts of the body, e.g. the hands, might also harbour the organism. The hands of a number of "non-milkers" were therefore examined by the same methods as for the milkers. The results are shown in Table 4.

Table 4

Case	Occupation	Treatment of hands before test scrubbing	Result: Lancefield Group B streptococci isolated from	
			Centrifuged deposit	Milk incubated with crystal violet
U	Laboratory worker	Hands washed with soap and water before test scrubbing	Negative	Negative
V	Laboratory worker	Hands not washed before scrubbing	Negative	Negative
W	Laboratory worker	Hands not washed before scrubbing	Negative	Negative
X	Teacher	Had just paid a visit to Farm 1 where streptococcal mastitis was known to exist. Hands not washed before scrubbing	Negative	Negative
Y	Farmer	Hands not washed before scrubbing	Negative	Negative
Z	Office worker	Hands not washed before scrubbing	Negative	Negative

As will be seen, in none of these cases were Lancefield Group B streptococci isolated, and it seems, therefore, that Lancefield Group B streptococci can be recovered only from the hands of those who milk cows regularly. It is, perhaps, worth recording that one of the laboratory workers was occupied in research on *Str. agalactiae* and the other two on dairy problems. The probability that the Lancefield Group B streptococci recovered from the hands of the milkers are *Str. agalactiae* is therefore strengthened. If this is so the milkers' hands are potential carriers of *Str. agalactiae* from cow to cow.

Colebrook & Maxted(1) have shown that soap and water properly used greatly reduce the hand flora and that various disinfectants (which, however, could not for obvious reasons be used during milking) still further reduce, if they do not eliminate, infection.

It may be that the small number of streptococci which have been isolated from the hands of milkers observing a routine method of hand disinfection would be insufficient to set up infection, but this cannot be asserted with any confidence.

It is tolerably certain, however, although it has not as yet been investigated, that in heavily infected herds, where no washing or disinfection of any kind is practised, the numbers of streptococci to be found on the milkers' hands would be considerably greater. The necessity for hand disinfection with adequate contact time is therefore obvious and, it may be assumed, present methods are inadequate. For the moment it may be suggested that proper scrubbing with soap and water should precede the use of a disinfectant, as several workers ((1) and (3)) have shown the benefits of the mechanical action of scrubbing in reducing the hand flora. Something in the nature of a detergent seems to be demanded, and for this purpose soap might be valuable. Experience at this Institute with hypochlorites as agents for the sterilization of dairy apparatus has shown that unless the surfaces are clean and "contact" real these substances cannot be relied upon for sterilization (9).

Although they are not at present advocated, the use of "milking fats" or substances of the nature of vanishing creams, containing some innocuous bactericidal substance for application to the hands, not the teats, might well be considered, in spite of the fact that they have been frowned upon on aesthetic grounds in this country in the past.

SUMMARY

Lancefield Group B streptococci have been recovered from the hands of milkers even in herds where infection was very light. Since these organisms had the biochemical reactions of *Str. agalactiae*, and since none were recovered from the hands of non-milkers, it is considered that they were identical with this organism and that the milkers' hands are a potential source of danger to cows. The methods of disinfection at present in use have been criticized and suggestions for improvements made.

The author wishes to thank Dr P. M. F. Shattock for the serological tests and Dr A. T. R. Mattick, who suggested these experiments, for his help and advice.

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(*MS. received for publication* 1 *November* 1940)

637.225:576.809.34

271. THE EXPERIMENTAL ERROR IN THE PLATE COUNT EXAMINATION OF BUTTER

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(With 2 Figures)

Consideration of the degree of accuracy with which bacterial numbers may be expressed is essential to the correct interpretation of the results of quantitative experiments or routine tests involving their estimation. Although considerable work has been done on the occurrence and significance of micro-organisms in butter, little attention appears to have been paid to the error of estimation of microbian populations in this product.

The total error in a count purporting to represent the bacterial population of a quantity of butter may be considered as the sum of the errors inherent in the actual technique of plating, and of the errors attributable to the irregularity of distribution of the organisms throughout the bulk of butter under consideration. The errors of technique are reflected in the degree of dispersion of parallel plate counts, which, under ideal conditions of plating, should conform to a Poisson distribution. If, as frequently happens with milk, the organisms present are aggregated in clumps which fail to become adequately dispersed throughout the dilutions and plating medium, gross discrepancies in parallel counts may be encountered. Under such conditions estimates based upon single plates or even the means of series of plates may be wholly unreliable. The sampling error in a fluid medium would be expected to be small, or at least capable of being controlled by accurate sampling methods. The process of manufacture and the physical nature of butter are such, however, that an appreciable degree of error in this respect would be anticipated.

This paper contains the results of an extended inquiry which was designed to throw some light on the nature of the distribution of bacteria in butter, and the accuracy of estimation of their numbers by the usual plate count technique.

Outline of preliminary investigation

During the last three years, 154 boxes of choicest commercial butter have been examined according to the following plan:

Plan of examination. Three cores of butter were taken from well separated portions of each box, the surfaces, to a depth of at least an inch, being neglected. The outside surfaces of each core were pared away with a sterile scalpel, and a 1 g. sample was transferred from the interior of the core to the first dilution blank with a sterile metal scoop designed to deliver this quantity. All dilution blanks were prepared by aseptically distributing $\frac{1}{4}$ strength

Ringer's solution in 9 ml. lots. Each sample was plated in triplicate in a dilution of 1/500 by transferring 2 ml. from a final 1/1000 dilution. As a check on the dilution, as well as on the plating and counting technique, the 1/100 and 1/1000 dilutions were also replicated in about half of the samples examined. Thus each plate in this particular series was prepared from individual dilutions which ranged back to a common 1/10 dilution. Nutrient agar to which 1% of skim milk had been added prior to the final sterilization was used throughout as a medium. The plates were incubated for 7 days at 30° C. They were counted in a light box with a tally counter and spotting pencil. Where the counts on a set of triplicate plates exceeded an average of 300 per plate, only one plate was counted. In these cases counting was done only on a representative area of one plate and the total estimated. Three workers participated in the examinations.

The χ^2 test

Where the averages of the three plates from a sample were below 300, χ^2 values were calculated from the formula $\chi^2 = \frac{S(x-\bar{x})^2}{\bar{x}}$. The frequency distributions of these χ^2 values are given in Tables 1 and 2 and are compared

Table 1. *Frequency distribution of χ^2 values.* 100 *sets of triplicate plates prepared from single dilutions*

χ^2 range ($n=2$)	Expected		Observed		χ^2
0	1		1		
0·020	1		1		
0·040	3	10	2	5	2·500
0·103	5		1		
0·211		10		9	0·100
0·446		10		7	0·900
0·713		20		23	0·450
1·386		20		20	0·000
2·408		10		8	0·400
3·219		10		13	0·900
4·605					
5·991	5		4		
7·824	3	10	5	15	2·500
9·210	1		0		
	1		6		
Totals	100		100		7·750

For $n=7$, 7·750 lies between 0·50 and 0·30 probability levels.

Table 2. *Frequency distribution of χ^2 values.* 208 *sets of triplicate plates prepared from replicated dilutions*

χ^2 range ($n=2$)	Expected		Observed		χ^2
0					
0·020	2·08		4		
0·040	2·08	10·40	3	9	0·188
0·103	6·24		2		
0·211	10·40			10	0·035
0·446	20·80			26	1·300
0·713	20·80			16	1·108
1·386	41·60			54	3·695
2·408	41·60			36	0·753
3·219	20·80			14	2·223
4·605	20·80			19	0·156
5·991	10·40			16	3·015
7·824	6·24		2		
9·210	2·08	10·40	2	8	0·554
	2·08		4		
Totals	208		208		13·027

For $n=9$, 13·027 lies between 0·20 and 0·10 probability levels.

with the expected distributions of χ^2. Further application of the χ^2 test shows that the departures from expectation are within the limits of random sampling in both tables.

The sums of the individual estimates of χ^2 were also tested, the values of $\sqrt{(2\chi^2)}-\sqrt{(2n-1)}$ being $+2{\cdot}83$ for the data of Table 1 and $-0{\cdot}32$ for Table 2. The test shows that in those samples in which the three plates were poured from a single final dilution, the error of estimation was abnormally high. The high value of $+2{\cdot}83$ appears to be a reflexion of the excess over expectation of χ^2 values above 9·21. This seems to indicate significant aberrations, due presumably to the unsatisfactory dispersal of clumped organisms. There is no evidence, however, of consistent gross errors due to this factor. As an indication of the general reliability of the means of multiple platings of butter samples, the agreement in the two series in question is regarded as encouraging.

Variation between samples within a box

The occasional occurrence of marked divergences in plate counts on different samples from a box of butter has been commented on by several investigators. The tendency has been to regard them as exceptional.

The present data provide information regarding the extent of variation actually experienced under normal routine conditions of plating, except that three plates were poured per sample and the estimates of total bacterial counts were the means of the counts on three plates.

Three samples were taken from each of the 154 boxes examined. In some boxes all three samples gave counts below 150,000 per g. (300 per plate), but in others, one, two or three of the samples yielded counts of up to several millions per g. In these cases the plates were uncountable in a 1/500 dilution, but since it was necessary to take all divergences into account, the colony numbers were estimated from a count of the colonies in a small area of one of the overcrowded plates.

Some difficulty has been encountered in presenting a picture of the variation encountered between samples within these 154 boxes of butter. The counts have finally been transformed to logarithms, and coefficients of variability calculated for the individual boxes. By this means the effect of wide variations in sample counts within boxes has been modified in the direction of reducing the apparent coefficient of variation. The distribution of the observed coefficients of variation calculated in this way is shown in Diagram 1.

The coefficients were found to range from 0·1 to 25 %. In the distribution of values, approximately 50 % of the figures obtained lay on either side of 4·0 %. A coefficient of variation of 4·0 % on a logarithmic scale would be represented, for instance, by counts of 14,500, 10,000 and 6900 per g. in the lower range of counts; or by 160,000, 100,000 and 63,000 per g. in the higher range. Approximately 10 % of the butters gave coefficients above 14·0 %. Typical counts at this level of variability would be 36,000, 10,000 and 2800 per g. in the lower range; 500,000, 100,000 and 20,000 per g. in the higher range.

Under routine conditions the bacterial count of a sample is commonly based upon the counting of one plate. This would have the effect of increasing the degree of between-sample variability. The author is indebted to Mr H. V. Rees of the Tasmanian Department of Agriculture for data which demonstrate the

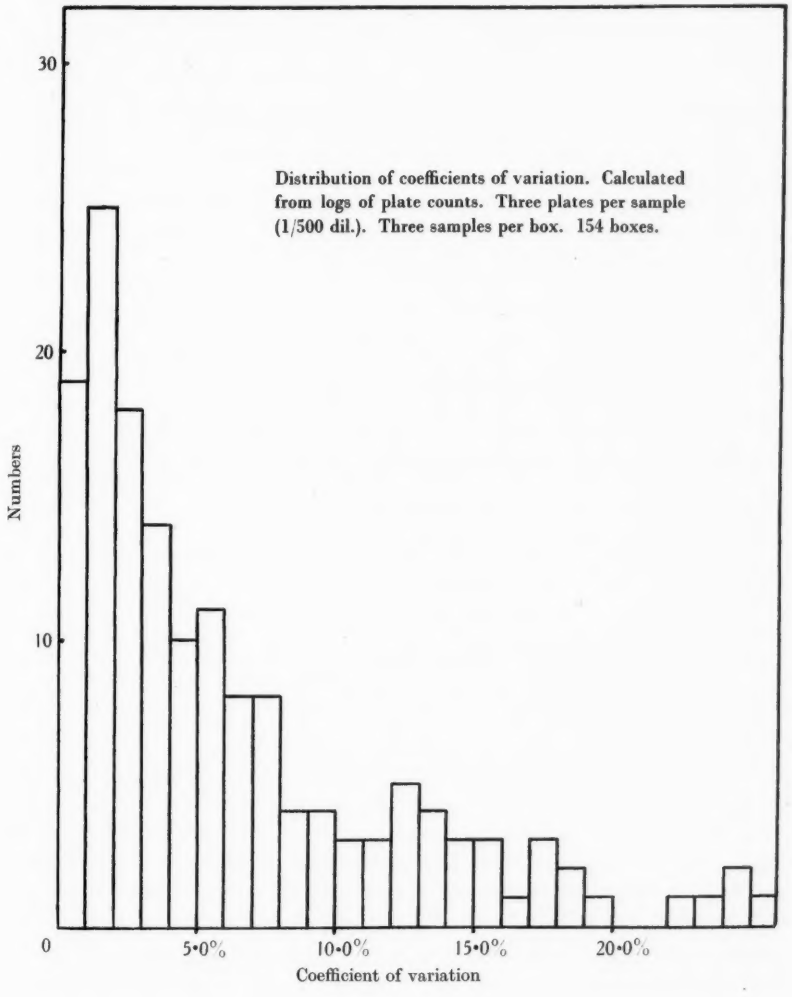

Diagram 1.

increase in variability when one plate only is counted. The technique of examination was otherwise identical with that employed in this laboratory. Fifty-eight boxes of butter were examined in this series. The relevant data are shown in Diagram 2. It will be seen that in this instance 50 % of the

coefficients fell on either side of a value of 6·0 % while 10 % had a value greater than 18·0 %.

The degree of variation at the upper limit is very striking. For instance, a coefficient of about 25 %, which occurred in about 2 % of cases over both series, is typified by sample counts of 900,000, 175,000 and 3500 per g.

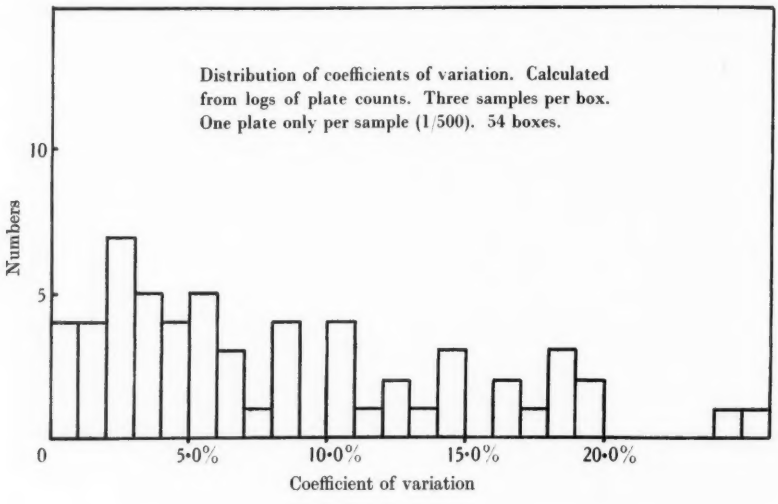

Diagram 2.

Sample variation in high quality butter

In considering the data available from the results of the foregoing examinations, it was thought that the degree of variation encountered between samples might have been influenced strongly by the following factors:

(*a*) The smallness in size (1 g.) of the samples taken.

(*b*) The possibility of gross sectional variations in the quality of butter throughout a box. This phenomenon is known by graders to occur frequently. It is obvious that if variations in quality do occur in this way, they might well be associated with large variations in bacterial counts.

A series of examinations was therefore planned in which precautions were taken only to use butter attaining a high standard of quality and uniformity. Furthermore, both the size of sample and the number of samples taken per box were considerably increased. If, by this means, the extent of between-sample variation could be so reduced that satisfactory estimates of box populations could be obtained, a starting-point would be available for the investigation of other types of butter, and of the suitability of less comprehensive methods of analysis.

Twelve boxes of butter were examined in this series. They each graded at least ninety-four points and were made by a group of three factories situated

in the same district under single control and using standard methods. The examinations occurred at intervals over a period of 6 months. The butters were approximately 7 days old at the time of examination. Seven samples were taken from each box, each sample being approximately 1 oz. in weight. This was melted down and, after thorough mixing, 5 ml. of melted butter were taken for examination.

Plan of experiment. From each box of butter seven samples were selected at random. The method of randomizing was as follows:

A plan of the area of the top of the box was drawn and divided into 1 in. squares. The outside inch was discarded, leaving an area of 10×10 in., which was divided into 100 squares. By taking two adjacent sides and selecting numbers from 1 to 10 from Tippett's tables as ordinates and abscissae it was possible to select squares strictly at random. Seven squares were selected in this way and their positions marked on the surface of the butter in the box. A trier was inserted at the points marked and a core of butter approximately 1 in.^{2} in section taken right through the depth of the box. Each core of butter was tested by a competent grader in order to detect, and eliminate if necessary, any boxes showing unevenness of quality. The top and bottom inch of the core were then discarded, and it was divided into two equal halves by cutting across the long axis of the core. The top or bottom half was selected as the sample by tossing a coin.

Bacteriological examinations. The outside of the sample was pared off with a sterile scalpel and a sterile $\frac{5}{8}$ in. test tube pushed through the core to a depth of 4 in., thus obtaining a cylinder of butter approximately 1 oz. in weight. This was released by gentle warming into a corked sterile 1 in. test tube. The sample was then melted at 40° C., thoroughly shaken and emulsified and 5 ml. transferred with a pipette to a dilution blank containing 45 ml. From each 1/10 dilution three separate 1/100 and 1/1000 dilutions were prepared. Plates were poured from each series of dilutions using milk agar as a medium. As each of the plates was made from an individual series of dilutions ranging back to the 1/10 dilution, tests for conformity of the final counts to the Poisson distribution could serve as a check on the general dilution and plating technique.

All dilution blanks were prepared from sterile Ringer's solution $\frac{1}{4}$ strength and were aseptically tubed. The plates were incubated for 7 days at 30° C. and were counted with the aid of the light box, spotting pencil and tally counter. The same worker conducted the whole series of examinations; twelve boxes of butter were examined, giving in all a total of eighty-four samples.

Chemical analyses. It was thought possible that irregularities in the distribution of micro-organisms throughout the butter might be correlated with variations in the moisture and salt contents. Chemical analyses for these constituents were therefore carried out on each sample.

The melted portion of butter from which the 5 ml. quantity was taken for the bacteriological examination was homogenized as thoroughly as possible by shaking in the rubber-stoppered 1 in. test tube and cooling down with tap water. Ten g. of butter were used for the moisture determination, which was performed by the rapid method, the butter being heated in a deep aluminium dish on a hot plate. As regards the salt determinations, 3 g. of sample were mixed with 10 ml. of hot distilled water and titrated with $N/10$ $AgNO_3$ using potassium chromate as an indicator.

Agreement with Poisson distribution

Of the eighty-four samples examined, thirty-nine gave plates countable (300 or less per plate) in the 1/100 dilution and forty-five in the 1/1000 dilution. The discrepancies between the three plate counts made on each sample were

again used to test the general agreement with the Poisson distribution. The values of $\sqrt{(2\chi^2)} - \sqrt{(2n-1)}$ are shown in Table 3, from which it will be seen that the value for the 1/100 counts significantly exceeded expectation (+1·87, with the 5 % point at 1·645).

Table 3. *Total χ^2 values calculated from triplicate plates counted in* 1/100 *and* 1/1000 *dilutions*

Dilution	No. of samples	$\Sigma(n)$	Total χ^2	$\sqrt{(2\chi^2)} - \sqrt{(2n-1)}$
1/100	39	78	102·53	+1·87
1/1000	45	90	85·83	−0·28
Totals	84	168	188·36	+1·11

The number of observations available is rather small for comparisons of the observed with expected distributions of χ^2, but these are shown in Table 4. Combining the end groups as shown by the brackets, the values of χ^2 for the agreement of the expected and observed distributions are 5·50 (P between 0·7 and 0·5) for the 1/100 counts and 11·00 (P between 0·2 and 0·1) for the 1/1000 counts. If the 1/100 and 1/1000 counts are taken together, giving eighty-four values, χ^2 is 6·63 with P between 0·5 and 0·3.

Table 4. *Frequency distribution of χ^2 values, calculated from triplicate plates counted in* 1/100 *and* 1/1000 *dilutions*

χ^2 range ($n=2$)	Sets counted in 1/100 dilution		Sets counted in 1/1000 dilution	
	Expected	Observed	Expected	Observed
0	0·39	0	0·45	0
0·020	0·39	1	0·45	1
0·040	1·17	0	1·35	1
0·103	1·95	2	2·25	4
0·211	3·9	6	4·5	1
0·446	3·9	3	4·5	6
0·713	7·8	6	9·0	13
1·386	7·8	8	9·0	9
2·408	3·9	2	4·5	1
3·219	3·9	5	4·5	2
4·605	1·95	2	2·25	4
5·991	1·17	1	1·35	2
7·824	0·39	2	0·45	1
9·210	0·39	1	0·45	0
Totals	39	39	45	45

$\chi^2 = 5{\cdot}50$, P between 0·7 and 0·5

$\chi^2 = 11{\cdot}00$, P between 0·2 and 0·1

Both taken together: $\chi^2 = 6{\cdot}63$, P between 0·5 and 0·3

The only significant departure from expectation shown by these tests was, therefore, the excessively high total χ^2 in the 1/100 dilution.

Analysis of variance

It so happened that, of the twelve butters examined, almost all the samples of six were counted in the 1/100 dilution and almost all the samples of the other six were counted in the 1/1000 dilution. The exceptions were two samples

counted in the 1/100 dilution in box 9, and five samples altogether counted in the 1/1000 dilution in boxes 3, 5, 8 and 10.

A detailed examination both of the error of estimation (variation within samples) and the variation between samples from the same box (variation within boxes) has been made by the method of analysis of variance. For this analysis the square roots of the plate counts were used for counts made in the 1/1000 dilution, and the square roots of one-tenth of the actual plate counts for samples counted in the 1/100 dilution; the figures thus represented the same quantities of butter. The mean squares both for within-samples and between-samples are shown for each box of butter in Tables 5 and 6.

Table 5. *Analysis of variance. Boxes in which majority of plates were countable in* 1/1000 *dilution. Square root transformation*

		Mean squares	
Box no.	Mean count per sample	Within-samples 14 d.f.	Between-samples 6 d.f.
7	25·6	0·1120	0·2507
11	24·7	0·2513	0·8286
12	24·4	0·3188	38·7715
9	18·3	0·2576	12·1979
4	17·3	0·1913	0·8968
1	9·3	0·3769	0·3154

Table 6. *Analysis of variance. Boxes in which majority of plates were countable in* 1/100 *dilution. Square root transformation*

		Mean squares	
Box no.	Mean count per sample	Within-samples 14 d.f.	Between-samples 6 d.f.
10	14·55	0·0522	19·2357
8	12·97	0·0738	2·7058
5	12·57	0·0603	8·2062
3	12·23	0·0367	9·7636
2	9·40	0·0224	0·1115
6	9·12	0·0226	0·6039

The estimates of variance from the different boxes were compared by Neyman & Pearson's L_1 test. For the within-sample variances in the six boxes counted at the 1/1000 dilution, $L_1 = 0{\cdot}933$ with the 5 % point at 0·873. There was thus no evidence that the six mean squares were not merely estimates of a common variance. They were therefore pooled, giving 0·2513 as the combined estimate of the within-sample variances in the 1/1000 dilution counts. The within-sample standard error of a single plating (error of estimation) was 0·5013. For the within-sample variances in the 1/100 counts, $L_1 = 0{\cdot}903$ with the 5 % point again at 0·873. The pooled estimate of variance was 0·0447, and the standard error of a single plating (error of estimation) was 0·2114.

Turning now to the between-sample mean squares, which are measures of the variation between the samples within each box, we find a very different situation. L_1 for the between-sample mean squares of the boxes counted at the 1/1000 dilution was 0·196 and of the boxes counted at the 1/100 dilution 0·378, with the 1 % point in both cases at 0·641. In each case the differences

between the six estimates are therefore highly significant. That is to say, even with high quality butter selected for uniformity, the variation within some boxes was of a very different order from the variation in others.

Correlation with moisture and salt content

The regressions of the bacterial counts on salt and on moisture content were calculated separately for each of the twelve boxes examined, the regression coefficients being as follows:

	Plate count/Salt	Plate count/Moisture
Box 7	+1·000	+3·633
11	−0·024	+0·853
12	+19·593	−121·100
9	+21·379	+8·583
4	+0·777	+2·750
1	−3·605	−3·207
10	−0·440	−31·783
8	+2·650	+10·438
5	−17·611	+33·118
3	+2·569	+7·755
2	−0·151	+0·474
6	−0·892	−3·833

Examination of the data indicates that there is no general correlation between the figures obtained for plate counts and those for salt and moisture content of the samples. This result is perhaps to be expected when it is considered that the determinations were made on relatively large quantities of butter. The figure for salt content, for example, is in effect the average of the salt contents of a large number of individual water droplets. The development of bacteria in the sample would be expected to be dependent, not on this average, but on the salt contents of the individual droplets. A few large droplets with a very low salt content may well raise the plate count of the sample markedly without affecting the average salt content of the sample to any significant degree.

Discussion

Fisher, Thornton & Mackenzie (1) have indicated that for the plate count to be a reliable estimate of the bacterial population of a sample, replicate counts must conform to the Poisson distribution. Tests of agreement with the Poisson distribution have been made for the data presented in both sections of this paper, and the evidence indicates that the plate counts were, in the main, reasonably satisfactory estimates of the bacterial content of the samples. However, the within-sample variability proved to be significantly in excess of that to be expected in the Poisson series both with the triplicate plates poured from a single 1/1000 dilution in the series of routine butters, and with the counts made in the 1/100 dilution in the series of selected butters. There is an indication therefore that, even with the careful technique employed in this work, the plate count was not always a reliable measure of the bacterial content of the sample.

A very wide disparity was frequently encountered throughout the investigation between the plate counts of different samples from the same box. When

three samples were taken per box under routine conditions the coefficients of variation of 50 % of the boxes were above 4·0 % on a logarithmic scale. In the series in which seven samples per box were examined, analysis of variance showed that the variability was of an extreme order in six of the twelve boxes examined. It must be emphasized that these twelve boxes were specially selected for high quality and uniformity, and that even less satisfactory results would be expected from the examination of poorer butters. When the possibility of encountering sectionally contaminated butter is also taken into account there appears to be little chance of obtaining a reliable mean count per box from the examination of samples small enough to be conveniently handled.

The bearing of these results on certain types of research problem are obvious. For instance, in experiments in which boxes of butter are treated as units, any correlation between bacterial counts and various factors associated with quality might quite likely be concealed by unevenness in the distribution of the organisms in the butter studied. In this type of problem it would appear that more accurate results would be secured if judgements of flavour or quality standards were determined only in respect of small samples, the bulk of these being used in the subsequent analyses.

Some observations can be made also in regard to routine examinations of butter samples. These are intended to serve mainly as a check on the general efficiency of factory sanitation. The handling of large numbers of samples for routine purposes requires a simple technique. It is, for instance, a common practice to base bacterial counts on one plate poured from one sample of butter. The foregoing data indicate that the result of a single plating does not give an accurate estimate of the bacterial population of a butter sample. Taken as an index of the mean bacterial population of a complete box of butter, it is very inaccurate and may be quite misleading.

It is only when a high estimate is encountered (e.g. several hundred thousand or more per g.) that any real degree of significance can be attached to the result. It would appear that errors arising from technique would be unlikely to influence a normally low count to this extent. The occurrence of butter giving rise to such a count, even though it appeared only in parts of a box or a churning, would, from the standpoint of quality control, indicate the need for remedial measures.

The correct interpretation of low counts is, however, made difficult, if not impossible, by the fact that the true mean count of the box might quite likely be considerably above or below the figure obtained for the sample. Under these circumstances the application of any rigid arbitrary standard as an index of factory contamination would lead to frequent anomalies. Although it would appear that inconsistencies could not be entirely eliminated by basing deductions on the results of multiple samples, preferably over a number of churnings, it is obvious that such a procedure would provide more reliable diagnoses.

SUMMARY

In a preliminary investigation into the experimental error of the plate count examination of butter, 154 boxes of choicest quality butter were examined by plating in triplicate, in a dilution of 1/500, each of three 1 g. samples per box.

χ^2 tests in both this and a later series indicated that the means of the triplicate counts in respect of plates averaging less than 300 colonies gave, in the main, reasonably satisfactory estimates of the bacterial content of the samples. In two of the tests, however, excessive within-sample variability was shown by abnormally high values obtained from the summation of χ^2. This is accepted as an indication that the plate count was not always a reliable index of the sample population.

The between-sample variability in this series has been shown by transforming the counts to logarithms and calculating the coefficient of variation in respect of each box. In the distribution of the coefficients approximately 50 % were found to lie on either side of a 4·0 % level, while 10 % gave values higher than 14·0 %.

In a further inquiry twelve boxes of butter were selected for quality and uniformity and data were secured from the examination of seven 1 oz. samples selected at random from each box.

Treatment of the results by the method of analysis of variance, using a square root transformation, indicated that the estimates of within-sample variance obtained could be regarded as estimates of a common variance. Highly significant differences were found, however, among the between-sample mean squares, and the variability was found to be excessive in six of the twelve boxes examined.

No significant general correlation was found in this series between bacterial counts and either salt or moisture determinations.

The application of these results to research problems and to routine tests involving the estimation of bacterial numbers in butter is discussed.

ACKNOWLEDGEMENT

The assistance of Mr F. C. McCleery in the statistical planning and analysis of these investigations is acknowledged. Thanks are also due to Mr A. M. Brown and other officers of the Dairy Branch, Department of Agriculture, who aided in obtaining samples and grading butter. The twelve boxes of butter used in the second section of the inquiry were placed at the author's disposal by Norco Co. Pty. Ltd.

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(*MS. received for publication* 25 *September* 1940)

637.333.1

272. "SLOWNESS" IN CHEESEMAKING

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Because of the widespread occurrence of "slowness" in cheesemaking in this country and elsewhere, much research has been carried out and speculation as to the possible causes of the trouble has been rife.

Whitehead (1) in New Zealand reported the presence of bacteriophage active against the lactic streptococci in cases where "slowness" had occurred. Whenever bacteriophage was recovered single-strain starters had been in use. These strains of bacteriophage were shown by Whitehead & Hunter (2) to be strain specific. Because of these reports incriminating bacteriophage as the cause of slowness it has been believed that it is also responsible for the occurrence of "slowness" in this country. As it is the practice in this country to use multiple strain starters, and as strain specificity, although not absolute, is displayed by these bacteriophages, it is improbable that they are a common cause of serious trouble. It is, nevertheless, possible for lysis of the dominant strain in the starter to occur with resultant slowness of acid production. Moreover, "slowness" which might result from the previous growth of "non-acid" streptococci or streptococci producing bacteriostatic substances in the cheese milk (Whitehead & Riddet (3)) has not yet been reported in this country. It has been shown by Davis & McClemont (4), and Mattick, Davies & Dearden (5) and stated by McDowall (6) that the inclusion of milk from cows suffering from mastitis in the bulk from which cheese is to be made has little or no effect upon the speed of acid production by the starter. The contrary is claimed by Leitch (7) and by Dibbern & Lembke (8). The presence of "abnormal" milk does, however, appear to have some effect upon the physical properties (texture and body) of the cheese (Davis & McClemont (4) and Johns *et al.* (9)), and upon the quality so far as flavour is concerned (McDowall (6)).

The first case of "slowness" investigated occurred at a farm in Dorset, where Cheddar cheese was being made, and was reported in the early summer of 1939. It appears that the "slowness" had been encountered annually, beginning early in May and continuing with varying severity till late July or early August.

On visiting the farm it was found that the milking herd consisted of about fifty cows of various ages, which were either South Devon or South Devon-Shorthorn cross. All milking was done mechanically, using bucket type milking units. Before milking each cow was tested with the strip cup for the presence of clots. Any animal showing abnormality was segregated and milked last.

All the usual precautions against bacterial contamination of the milk were taken. The cheese were made from mixed evening's and morning's milk by an experienced cheesemaker. The evening's milk was cooled in the vat by water circulation.

The final cheese produced were usually of very good quality even when the "slowness" was at its height. The curd during manufacture was often unsatisfactory, particularly when it was transferred from the vat to the cooler. At this stage it became weak and developed holes. Co-inciding with this change the fall in titratable acidity which is noted below occurred.

Typical records of working times and acidities are shown in Table 1, and it will be seen from these figures that definite "slowness" was present, although not as severe as later in the investigation.

Table 1. *Manufacture data at commencement of investigation*

Time of observation	Date of manufacture: 4. vi. 39	†	6. vi. 39	†	7. vi. 39	†	8. vi. 39*	†
Temp. of bulk milk, a.m.	83° F.		70° F.		71° F.		74° F.	
Time starter added	6.45 a.m.	—	7.15 a.m.	—	6.55 a.m.	—	7.5 a.m.	—
Time of rennetting	9.43 a.m.	0.00	9.35 a.m.	0.00	9.45 a.m.	0.00	9.51 a.m.	0.00
Acidity at rennetting	0·19	—	—	—	0·185	—	0·19	—
Time of beginning of cut	10.21 a.m.	0.38	10.05 a.m.	0.30	10.20 a.m.	0.35	10.24 a.m.	0.33
Temp. of scald	101° F.	—	102° F.	—	102° F.	—	102° F.	—
Time steam turned off	11.24 a.m.	1.41	11.15 a.m.	1.40	11.25 a.m.	1.40	11.20 a.m.	1.29
Time of pitching	11.41 a.m.	1.58	11.34 a.m.	1.59	11.35 a.m.	1.50	11.49 a.m.	1.58
Acidity at pitching	0·19	—	0·165	—	0·19	—	0·18	—
Time when put on cooler	12.25 p.m.	2.42	12.35 p.m.	3.00	12.29 p.m.	2.44	12.48 p.m.	2.57
Acidity of whey from cooler	0·31	—	0·29	—	0·34	—	0·35	—
Time milled	3.55 p.m.	6.12	4.13 p.m.	6.38	4.10 p.m.	6.25	3.45 p.m.	5.54
Acidity at salting	0·84	—	0·70	—	0·79	—	—	—

* 1·5 oz. $CaCl_2$, dissolved in 1 pint of water, added to 130 gal. milk at 9.50 a.m.

† Figures in this column show time in hours and minutes from addition of rennet.

Of the possible causes of the "slowness" the following were investigated:

(*a*) The effect of the inclusion of a quantity of mastitis milk.

(*b*) The presence in the milk of "non-acid" organisms.

(*c*) The presence of bacteriophage active against the starter in use.

(*d*) Bacteriological abnormalities of the milk other than the above.

Bulked samples from all four quarters of each cow were taken and tested for mastitis, using the routine of Edwards (10). It was found that only two cows were suffering from streptococcal mastitis, although nine others appeared to have staphylococcal infections, revealed by plating on a blood medium without crystal violet. The milk from these eleven cows was on several occasions kept separate from the bulk milk and made into cheese under conditions exactly similar to those applying to the bulk "normal" milk. The data for these cheese are given later.

Attempts to isolate the non-acid organisms described by Whitehead & Riddet (3), using their technique, were uniformly unsuccessful.

Again using the technique of Whitehead & Cox (11) six different strains of lactic streptococci, isolated from the starter in use at the farm, were tested with whey filtrates, collected at varying times throughout the process of manufacture on several days, for the presence of bacteriophage. No positive results were recorded, although repeated efforts were made to demonstrate its presence by the method of "feeding and filtering".

Bacteriological examinations of the bulk milk were made on several days by plating on yeastrel agar (Table 2). On two occasions the total count for the evening's bulk milk was comparatively small, and the increase which had taken place by the morning is readily understandable when it is seen that the temperature of the milk in the vat in the morning was usually over 70° F.

Table 2. *Numbers of "miscellaneous" organisms per ml. on yeastrel agar*

				Count			Count	
Sample	Date	Count	Date	Clean milk	Mastitis milk	Date	Clean milk	Mastitis milk
Bulk milk, p.m.	6. vi. 39	4,000	12. vi. 39	<1,000	<1,000	14. vi. 39	500,000	100,000
Bulk milk, overnight	7. vi. 39	2,500,000	13. vi. 39	2,000,000	200,000	15. vi. 39	3,000,000	3,000,000
Whey at pitch	7. vi. 39	10,000,000	13. vi. 39	—	—	15. vi. 39	8,500,000	8,000,000
Whey at draw	7. vi. 39	12,000,000	13. vi. 39	—	—	15. vi. 39	6,000,000	6,000,000
Whey from cooler	7. vi. 39	11,000,000	13. vi. 39	—	—	15. vi. 39	—	—

In order to estimate the approximate numbers of each of the types of organisms present the random selection plate, described by one of the authors (12), was used. These results are given in Table 3. The composition of this flora was in no way abnormal. In two cases the coliform population is high, but this is not unusual in some cheese milks.

Table 3. *Composition of bacterial population*

		Micrococci		Lactic streptococci		Coliform		Other rods		Miscellaneous	
Date of sampling	Total no. isolated	No.	% of total	No.	% of total	No.	% of total	No.	% of total	No.	% of total
7. vi. 39	21	7	33·33	4	19·05	4	19·05	4	19·05	2	9·52
13. vi. 39	23	5	21·73	4	17·40	5	21·73	6	26·10	3	73·04
15. vi. 39											
Clean milk	14	4	28·57	3	21·45	2	14·28	4	28·57	1	7·15
Mastitis milk	11	5	45·50	3	27·50	1	9·00	1	9·00	1	9·00

All lactic streptococci, isolated either from the starter or from the milk, were tested and grouped according to their biochemical reactions (13). These results are given in Table 4. The flora of the starter was mainly *Str. lactis*; this

Table 4. *Composition of lactic streptococcal flora*

		Str. lactis		"Intermediate"		*Str. cremoris*	
Source	Total no. isolated	No.	% of total	No.	% of total	No.	% of total
Starter	45	23	51·10	11	24·45	11	24·45
Milks	14	5	35·70	6	42·90	3	21·40

is unusual, because normal starters contain a larger proportion of *Str. cremoris* than *Str. lactis*. The streptococci isolated from the milk showed, however, a very even distribution through the groups.

Breed smears of the whey, collected at various times during the process of manufacture, were examined (Table 5) and there appears to have been little, if any, increase in numbers over the period of examination. This fact probably only reflected the condition of "slowness". The cause of the failure to increase in numbers was, at the time of the experiment, not clear, but upon investigation of the second case of "slowness" reported below a possible explanation was arrived at.

Table 5. *Numbers of bacteria present in the whey (estimated by Breed smear method)*

	Date of manufacture			
Sample	8. vi. 39	12. vi. 39	14. vi. 39 Clean milk	14. vi. 39 Mastitis milk
Whey at pitching	10,000,000	17,500,000	12,800,000	11,200,000
Whey at drawing	—	—	11,000,000	11,700,000
Whey from cooler	12,500,000	16,000,000	19,000,000	19,000,000

It was noted earlier that there was a tendency for holes to form in the curd when transferred from the vat to the cooler. Attempts were made to isolate any organisms which might be responsible for this apparent production of gas by taking cultures from around the holes and examining the flora so obtained. This gave negative results.

At short intervals throughout the process of cheesemaking observations were made of the titratable acidity and the *p*H of the whey, and in some cases the *p*H of the curd was determined at the same time. These results are given in Tables 6 and 7.

Table 7. *Showing p*H *of whey and curd during manufacture*

		Date of manufacture				
		12. vi. 39	13. vi. 39		15. vi. 39	
Period when sampled		Bulk	Clean	Mastitis	Clean	Mastitis
At pitching	Whey	—	—	—	5·64	5·76
	Curd	—	—	—	5·47	5·62
At beginning of draw	Whey	5·67	—	—	5·57	5·67
	Curd	5·43	—	—	5·32	5·34
2nd cut or curd cut in fingers	Whey	5·28	5·18	5·25	—	—
	Curd	5·26	5·11	5·28	—	—
On cooler, 1st sample	Whey	5·67	5·54	5·33	5·77	5·27
	Curd	5·17	5·18	5·20	5·08	5·15
On cooler, 2nd sample	Whey	5·76	†	†	†	†
	Curd	5·10	—	—	—	—
On cooler, 3rd sample	Whey	†	—	—	—	—
	Curd	5·05	—	—	—	—
At salting	Whey	4·93	—	—	—	—
	Curd	4·88	5·08	—	—	—

† No more whey off cooler.

The fall in acidity mentioned earlier is clearly shown by these figures, and it appears to have occurred in nearly all cheese as soon as the curd was transferred from the vat to the cooler. This is also shown in the *p*H values for the whey, although there seems to have been a slow but steady rise in the acidity of the curd during the period on the cooler. The same fall in acidity occurred even when the "mastitis" and "non-mastitis" milk were kept separate. It was again observed at a later stage of the investigation when, by changing the strain of starter, the "slowness" had apparently been eradicated (Table 8). It was shown by testing that this fall in acidity was not due to the presence of excess alkali on any of the utensils in use.

Table 8. *Observed fall in acidity with changed starter*

Date of making	Highest acidity reached before putting on cooler	Lowest acidity recorded on cooler	Fall in acidity
16 June	0·405	0·31	0·095
17	0·34	0·21	0·13
18	0·365	0·30	0·065
19	0·46	0·30	0·16
20	0·47	0·21	0·26
21	0·47	0·45	0·02
22	0·45	—	—
23	0·41	—	—
24	0·49	0·45	0·04
25	0·47	0·40	0·07
26	0·54	0·44	0·10
27	0·49	0·36	0·13
28	0·345	—	—
29	0·40	—	—
30	0·47	0·42	0·05
1 July	0·54	0·34	0·20
2	0·47	0·44	0·03

Apart from this it appeared that the acidities encountered when working with the mastitis milk were in all cases higher than those of the control non-mastitis milk. The working times for these cheese are given in Table 9. The times recorded are still slow for both milks and the exclusion of the "mastitis" milk appears to have had no beneficial effect.

In the absence of evidence that the milk itself was the cause of the "slow" starter trouble, it was thought that the starter might be at fault. With this possibility in view an entirely fresh strain of starter was procured and various trials carried out with it.

Starter *A* was obtained from a different source from any used on the farm and was cultured in the milk of the farm.

Starter *B* was the one already in use on the farm. It had been freshly procured only a week before the investigation was begun and had never been "fast". It came from the same source as one which had "gone slow" some time previously.

Starter *C* was the same as starter *A* except that it was cultured in milk other than that of the farm.

These starters were cultured separately at various times in both raw and pasteurized milk. The results of these experiments are given in Table 10.

Table 9. *Manufacture times and acidities of "mastitis" and "non-mastitis" milks*

Observations	Date of manufacture: 13. iv. 39 Non-mastitis		13. iv. 39 Mastitis		14. iv. 39 Non-mastitis		14. iv. 39 Mastitis		15. iv. 39 Non-mastitis		15. iv. 39 Mastitis	
Acidity of milk p.m.	0·16	*	0·16	*	0·16	*	0·16	*	0·17	*	0·165	*
Time starter added	6.55 a.m.	—	6.55 a.m.	—	6.58 a.m.	—	6.57 a.m.	—	6.47 a.m.	—	6.48 a.m.	—
Time of rennetting	9.46 a.m.	0.00	9.46 a.m.	0.00	10.10 a.m.	0.00	10.20 a.m.	0.00	9.32 a.m.	0.00	9.38 a.m.	0.00
Acidity at rennetting	0·19	—	0·19	—	0·21	—	0·205	—	0·195	—	0·185	—
Time of cut (beginning)	10.20 a.m.	0.34	10.21 a.m.	0.35	10.37 a.m.	0.27	10.45 a.m.	0.25	10.09 a.m.	0.37	10.20 a.m.	0.42
Temp. of scald	104° F.	—	104° F.	—	104° F.	—	104° F.	—	104° F.	—	104° F.	—
Time of pitching	11.47 a.m.	2.01	11.56 a.m.	2.10	12.00 n.	1.50	12.17 p.m.	1.57	11.55 a.m.	2.23	12.00 n.	2.22
Acidity at pitching	0·20	—	0·20	—	0·24	—	0·24	—	0·215	—	0·195	—
Time when put on cooler	12.56 p.m.	3.10	12.58 p.m.	3.12	12.47 p.m.	2.37	12.53 p.m.	2.33	1.05 p.m.	3.33	1.05 p.m.	3.27
Acidity of whey from cooler	0·37	—	0·47	—	0·36	—	0·48	—	0·48	—	0·48	—
Time milled	5.15 p.m.	7.29	5.50 p.m.	8.04	3.55 p.m.	5.45	4.05 p.m.	5.45	5.05 p.m.	7.33	5.15 p.m.	7.37
Acidity from press	0·83	—	0·92	—	0·63	—	0·88	—	0·83	—	0·87	—

* Figures in this column represent time in hours and minutes from time of rennetting.

The use of starter *A* and starter *C*, either alone or in conjunction with starter *B*, at first had no beneficial effect on the time occupied in cheesemaking. It seems likely that a period of time was necessary to "acclimatize" starter *A* to the milk. Later when "acclimatization" had taken place the use of starter *A*, either by itself or in conjunction with starter *B*, shortened the making time very appreciably. In order to ensure that this apparent cure was not due to the spontaneous disappearance of the trouble, starters *A* and *B* were used on alternate days. "Slowness" returned immediately when starter *B* was used alone.

It appears from the data already given that the chief factor responsible for the "slowness" was the starter itself; change of starter strain resulted in a more normal rate of acidity development. There is as yet no concrete evidence to account for this, although various suggestions may be advanced.

Table 10. *Manufacture times with change of starters*

Date of manufacture	Starter used	Time from rennetting to press	Acidity at press
16 June	*B* past.*	7 hr. 50 min.	0·80
17	*A* past.	7 52	0·87
18	*C* past.	8 0	0·83
19	*C* past.	7 0	0·84
20	*C* past. + *B* raw†	8 20	0·84
21	*A* + *B* raw	5 15	0·84
22	*A* + *B* raw	5 15	0·85
23	*A* + *B* past.	5 30	0·87
24	*A* + *B* past.	5 40	0·78
25	*B* past.	7 30	0·87
26	*A* past.	5 50	0·90
27	*A* + *B* past.	5 30	0·91
28	*B* raw	6 0	0·82
29	*A* raw	5 45	0·89
30	*B* raw	8 10	0·99
1 July	*A* raw	5 25	0·93
2	*A* raw	5 50	0·92

* past. = starter cultivated in pasteurized milk.
† raw = starter cultivated in raw milk.

It may be assumed that the starter on this farm had become a single-strain starter composed of *Str. lactis*. The final "slowness" may therefore have been due to "phaging" to which single-strain starters appear to be particularly susceptible, and although the presence of "phage" was never actually demonstrated, the possibility of its presence must be admitted.

Owing to the use of multiple-strain starters in Britain it is difficult to demonstrate a "phage" active against one or more strains of streptococci where many different strains are present. It may be that in the very early stages a "phage" lytic for one of the active strains of *Str. cremoris* in the starter was actually present. The elimination of the active strain might have engendered the appearance of a resistant, less active, strain which was finally replaced by the apparently more phage-resistant *Str. lactis* types. Such an effect might continue to occur as long as starters which contained strains, susceptible to a phage present at the farm, were used.

In a second case where "slowness" was encountered the starter was tested for acid production at different temperatures, and compared with the behaviour of two control starters. A typical series of readings is given in Table 11.

Starter *S*, the one under observation, had a normal acid production at temperatures up to 37° C., but failed to produce acid at 40° C. Starter *X* produced acid at 40° C. and starter *Y*, although producing less acid than starter *S* at 37° C., was still capable of producing acid at 40° C., although the quantity was considerably less than that produced by starter *X*. All three starters failed to grow at 45° C., showing incidentally that no strains of *Str. faecalis* were present.

Again, after 24 hr. incubation at 40° C. starter *S* was incapable of further growth either at 22° or 28° C. Starters *X* and *Y* on the other hand were capable of growth at the lower temperatures after 24 hr. incubation at 40° C. Starter *S* was therefore unable to resist the higher temperature conditions found at scalding.

In the light of this it seems evident that the use of a starter capable of normal growth at 40° C., which should be a property of every starter, and

Table 11. *Titratable acidity of starters incubated for* 24 *hr. at various temperatures*

	Acidity of starters expressed as ml. *N*/9 NaOH per 10 ml. culture					
Starter	22° C.	28° C.	30° C.	37° C.	40° C.	45° C.
Starter *S*	10·20	10·00	9·90	4·70	0·00	0·00
Starter *X*	10·00	—	10·00	6·40	5·80	0·00
Starter *Y*	10·20	9·60	10·00	3·50	2·80	0·00

has been advocated by Whitehead & Cox (14), would in some cases overcome the difficulty of "slowness".

A mixed starter culture composed of starters *X* and *Y* was dispatched for trial under practical conditions at the place where the "slowness" was occurring. When this starter was used the trouble disappeared and after many weeks the recurrence of "slowness" had not been reported.

It is not claimed that the inability of a starter to grow at 40° C., or even higher, is the only cause of "slowness" in this country, as there would appear to be other factors concerned; for example, bacteriophage has not yet been completely excluded. In both cases detailed above "slowness" had been experienced some considerable time before these investigations began, and fresh cultures of the starters had been procured from the same source as the ones which had previously gone slow; these new cultures also went "slow" soon after arrival. In spite of this, however, no trouble was experienced with the "mother" cultures of either of these two starters at their place of origin when used for cheesemaking. It seems, therefore, that there may be some factor in the milk, or the method of starter propagation, which encouraged strains of streptococci incapable of continued acid production at a scalding temperature of 40° C. Thus a lag in acid production may take place when the

temperature is raised and continue until the conditions in the curd again permit the resumption of acid production. It cannot be stated what the local factors were, but that they existed, in one instance at least, is borne out by the fact that starter *A*, in the first case reported, when first used on the farm did not improve working times, and it was not until some generations had been cultivated in the milk that any improvement was noticed. This lag in activity had been referred to earlier as "acclimatization".

The fall in the acidity in the whey when the curd was transferred to the cooler has not as yet been accounted for. Whether or not this is a normal effect still remains to be proved.

SUMMARY

Two cases of "slowness" in cheesemaking are reported in which the trouble was eliminated by changing the source of the starter.

Attempts to isolate either bacteriophage or "non-acid" organisms failed.

Abnormal milk had no effect on the rates of acid production when compared with control normal milk.

A fall in the acidity of the whey draining from the cooler was observed.

The inability of certain strains of streptococci to grow at scalding temperatures appears to be one cause of "slowness" in cheesemaking.

We wish to record our thanks to Mr T. R. Ferris, Dorset County Council, for drawing our attention to this problem and to the owners of the farm and dairy for giving us every facility for carrying out these investigations. We are also grateful to Dr P. M. F. Shattock for the mastitis tests and to Dr A. T. R. Mattick for his help and advice.

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(*MS. received for publication* 20 *July* 1940)

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273. THE CONSISTENCY OF CHEESE CURD AT THE PITCHING POINT AND ITS BEARING ON THE FIRMNESS AND QUALITY OF THE FINISHED CHEESE

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(With 3 Figures)

Introduction

In an earlier paper(1) a method was described for measuring what is, in fact, the superficial density of cheese curd at the pitching point under standard conditions, and preliminary experiments were quoted suggesting that this test should give a useful practical measure of the consistency of curd at this stage of cheese manufacture.

Many further experiments have now been carried out using the method but with a somewhat more compact and portable form of apparatus.[1] One slight improvement in technique has also been effected which makes for less hurry in doing the weighing. The second inversion of the samples is now made at 15 instead of 20 sec. from the start, which gives an extra 5 sec. (i.e. 12 sec. in all) to prepare for the weighing. Comparative tests using both methods show no important difference in the figures obtained.

In the present paper, data will be presented to strengthen the case for the practical utility of the test, to study the self-consistency of a single cheesemaker during a period of about nine months, to consider the efficacy of the method for comparing techniques in different factories and, finally, to relate consistency figures to data obtained by a new apparatus for measuring the body of finished cheese. Work was carried out primarily at the British Dairy Institute, Reading (factory A) and at the Somerset Farm Institute, Cannington (factory B). A number of data was also obtained from two factories belonging to Messrs Aplin and Barrett (factory C, pasteurized milk, and factory D, raw). In view of its primary importance in the Southern Counties (especially in war time) most of the tests were done on Cheddar, though in factory A further data were also obtained for all the other varieties mentioned in the earlier paper.

Cheddar, Factory A

In the earlier paper it was tentatively suggested that a value of W/h of about 19·0 is the most suitable for normal Cheddar curd in the technique used in this factory. We are now in a position to substantiate this more fully. Fig. 1 shows

[1] This apparatus may be obtained from Messrs Cope and Cope, King Street, Reading. A small number are available on loan for experimental purposes and may be borrowed from the National Institute for Research in Dairying.

a histogram giving the number of cheese pitched within each interval of 0·2 during the period under review. The black squares indicate a judgement of "normal" on the part of the expert for normal acidity. "Soft" judgements are marked with crossed lines and "firm" with broad hatching. It is apparent that the median for "normal" judgements lies at 19·3, for "firm" at about 17·7 (very few data) and for "soft" judgements at 19·7, the means being 19·0,

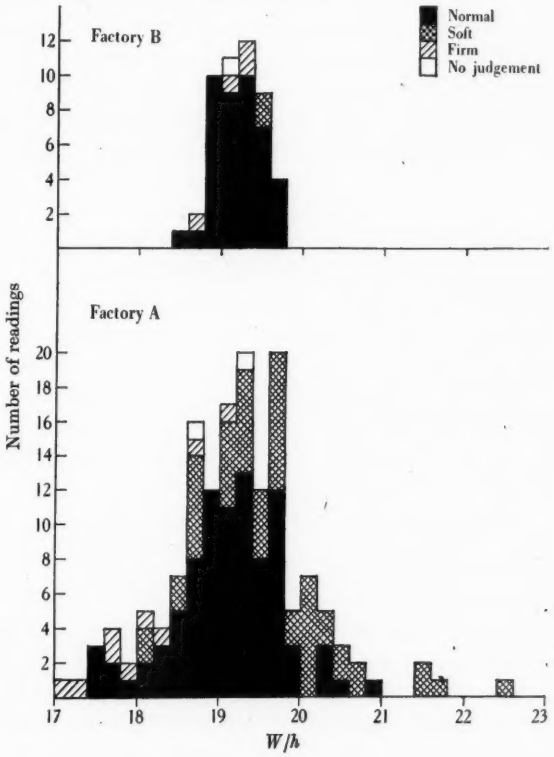

Fig. 1.

17·9 and 19·6 respectively. The distribution is remarkably regular though not quite even, there being relatively more values on the firm side than on the soft side of the mode.

In studying the variations in firmness of curd at cutting during a period of about a year, Coppen (2) found that in the summer months the cheesemaker quite unwittingly tended to cut softer than in the winter. In the present case, during early summer, the cheesemaker gave it as her opinion that she was pitching softer than at earlier and later periods, but the mean values for the three periods for Cheddar, 19·0, 19·2 and 19·0, do not differ significantly.

As pointed out in the earlier paper, it can never be finally proved that our

W/h figure really measures the mixture of physical properties judged by the expert in handling the curd. Strong evidence is now available, however, that this assumption is justified, since it is found that for the expert in factory A, the smallest percentage difference in W/h of curd which she can judge correctly more frequently than can be accounted for by chance (threshold) is lower than is the case for truly fluid materials (bitumens) for the same person.[1] Since at this level, this expert is exceptionally good at judging elastic properties, the threshold for cheese curd works out almost as low as that for rubber for an average person. This is truly remarkable. Not only does it show that W/h is an excellent criterion of the properties assessed by this expert but it also suggests, as one might have expected from physical considerations, that it is by their elastic rather than by their plastic properties that the curds are being judged.

Acidity

The cheesemaker in factory A consciously attempts to pitch softer for sweeter and firmer for more acid curds. Judgements were always given to us as if for a normal acidity, so that in the case of a sweet curd, for example, the expert would report the curd as normal for such an acidity, but softer than normal had the curd shown a normal acidity. Such a judgement would be recorded as "soft". There should therefore be a correlation between these recorded judgements and the acidities. Whereas for the purposes of Fig. 1 only three categories were recognized, in the present case the more detailed judgements were recorded and scored thus:

Very firm	Firm	Slightly or rather firm	Very slightly firm	Normal	Very slightly soft	Slightly or rather soft	Soft	Very soft
1	2	3	4	5	6	7	8	9

There was no apparent correlation between W/h and acidity, and also in the case of the subjective judgements and acidity the correlation coefficient was quite insignificant. There is some evidence, however, that in extreme cases the subjective firmness may be correlated with acidity, but further data are required to substantiate this. The expert in factory B expressed the opinion that, in trying to predict the W/h values for curd from feel (which she did with remarkable accuracy) very acid curds tended to give the impression that W/h was considerably higher than experiment afterwards showed to be the case. It is possible here that factors other than those associated with superficial density are taken into account.

Comparisons of W/h values for Cheddar in different factories

That the techniques used in factories A and B are by no means identical is clear from Table 1. In view of this fact, it is all the more remarkable that the histograms for the two factories should be so similar (Fig. 1). The medians

[1] The psycho-physical aspects of these experiments will be discussed elsewhere.

Table 1 (*Average values*)

	Temperature of scald	Time of scald (minutes after cutting complete)	Cut*	Acid at pitching
A	102°	59·1	Normal	0·174
B	99°	71·5	Rather fine	0·131

* Taking A as standard.

are 19·3 and 19·3 for A and B respectively and the means 19·0 and 19·2 (normal judgements). There were, of course, far fewer data available for B.

In factory C, the situation is quite different. Here the expert cheesemaker was trained in Scotland and prefers a much firmer curd at pitching. Moreover, the milk is pasteurized. The conditions in factories C and D are approximately as follows:

Table 2

	Temperature of scald	Time of scald	Cut	Acid at pitching	Mean W/h
C	104–105°	70 min.	Normal	0·19	15·2
D	104°	70 min.	Normal	0·20	16·3

Raw milk is used in factory D.

At present only a small number of data is available for these two factories, but there are sufficient to show the marked differences involved, in spite of similarity of technique.

Cheese other than Cheddar

A considerable number of new data has been collected at factory A for cheese of various varieties. Where these were adequate for the construction of a histogram they are given in Fig. 2. It must be remembered, however, that the primary function of factory A is teaching and that for this purpose a large variety of cheese has to be made. Although the experts are highly skilled, it is not possible for them to have the advantages of steady routine production of every type of cheese made there. It has also been made clear already that there are marked differences in technique in different factories in making cheese purporting to be of the same type. It would therefore be incorrect to interpret the medians of the histograms in Fig. 2 as the only *correct* values for the types of cheese in question. They are nevertheless typical for the techniques found effective in producing good cheese in factory A.

In comparing different types of cheese, one is naturally interested in the way in which the moisture is distributed within the particles of curd. Although generally speaking the higher the W/h figure the higher the moisture content, the size of cut and the extent to which the whey is entrapped in the curd by rapid scalding have a marked effect on the ratio of these properties.

As already stated(1) reproducibility in moisture data from curd at the pitching point is not good, the moisture content histograms having a wide

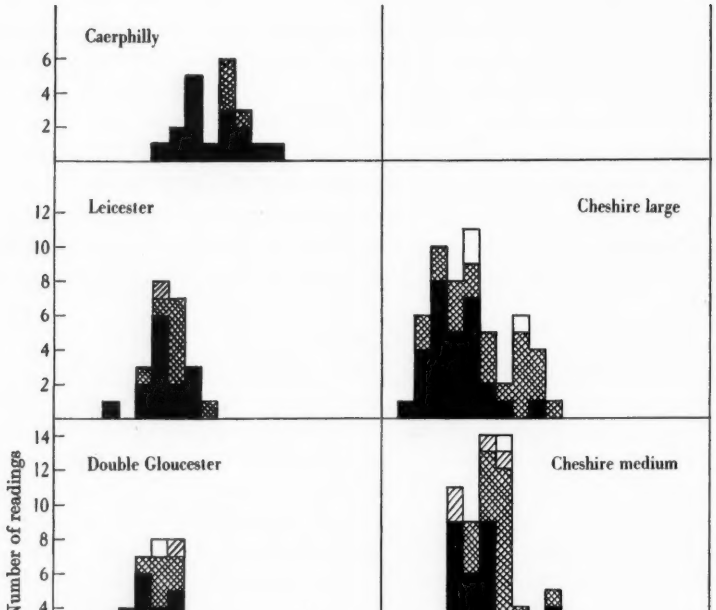
Caerphilly
Leicester
Cheshire large
Double Gloucester
Cheshire medium
Derby
Cheshire small
Number of readings
W/h
17 18 19 20 21 22 23 24 25

Fig. 2.

scatter, but it will be seen from Table 3 that variations in $\frac{W/h}{\text{moisture}}$ differ markedly for different types of cheese (factory A).

Table 3

Cheese type	Median of $\frac{W/h}{\text{moisture}}$ data*
Cheddar	0·33
Cheshire: Large	0·29
Medium	0·30
Small	0·31
Derby	0·29
Caerphilly	0·30
Double Gloucester	0·31
Leicester	0·31

* Some of these data are derived from rather a small number of cheese and should be regarded as provisional.

It is interesting to note that in the case of Cheshire and Derby cheese at factory A there was, during the period when the expert believed herself to be pitching soft, a definitely higher value of W/h than before and after this period (Table 4). This is especially true in the case of the large cheese (60 lb.), where the expert predicted that the greatest differences would occur.

Table 4. *Medians for 1st, 2nd and 3rd periods of experiment*

	1st period		2nd period		3rd period	
	Med.	Mean	Med.	Mean	Med.	Mean
Cheshire: Large (60 lb.)	18·8	18·8	19·7	19·6	18·4	18·3
Medium (30–40 lb.)	20·2	20·1	22·2	20·6	19·3	19·4
Truckle (9 lb.)	21·2	21·1	22·2	21·2	21·3	21·1
Derby	21·3	21·3	23·2	22·4	21·8	22·3

As already stated, the figures for Cheddar did not show a significant difference.

A comparison between moisture data from factories A and B presented some difficulties, since it was not possible to carry out moisture determinations at B. It was found, however, that samples taken at A and kept in suitably waxed-sealed specimen tubes for a week yielded results which differed but little from those taken in the ordinary way and tested immediately. By means of the same technique of sealing, samples were collected at B and analysed some days later at A. Although the accuracy of this procedure is not as high as is to be desired, it seems likely that the differences in median for Cheddar with respect to moisture content are significant. For A, the median is 59 % and for B 56 %. This may well be accounted for by the difference in size of cut in the two factories.

Relation of W/h values to body of finished cheese

The whole practical significance of measurements of pitching consistency depends on the supposition that, for any given technique, it is most important to pitch at the right firmness, and that if this is not done, the quality of the cheese will suffer.

It is of immediate importance to inquire how big a variation in W/h can be tolerated without serious change in the body of the finished cheese. But first, a quantitative measure of body is required. For this purpose, it was thought best to use methods imitating as nearly as possible the subjective tests of the cheese grader rather than to use the more accurate methods of Davis (3), although these latter are much to be preferred for other purposes.

Two tests were devised. In one, a standard cheese skewer was fitted with a spring handle and inserted into the cheese in the usual way, the force exerted being recorded from the contraction of the spring. It was found that unless the skewer was inserted very slowly or very fast, a reproducible figure could be obtained without any special precautions as to speed of insertion. Moreover, once the broadest part of the blade has passed the rind the reading remained constant. It was this steady reading that was recorded (S). The much smaller resistance offered by the rind itself could be measured if required.

The second apparatus was designed to imitate the pressure of the thumb on the top of the cheese. The apparatus is shown in Fig. 3.

The compressor A consists of a part of a sphere (diam. 1·5 in.) made of metal. The load is applied by giving a steady pressure on the handle B, steadying the plate C (diam. 6 in.) flat on the surface of the cheese near the centre with the other hand. The pressure applied is controlled by the spring. The collar D is set so that it makes contact with the stop E when the required pressure (36 lb. is convenient for Cheddar) is reached. Care must be taken not to exert pressure on the stop, since any such pressure would be in excess of that required. Pressure is released within about 0·5 sec., the grub screw F is tightened and the apparatus lifted to read the deformation in the cheese after the removal of pressure ($\epsilon_{\text{plastic}}$) directly on the vernier G. During the application of the pressure, when the cheese is undergoing maximum deformation, the sleeve H (initially resting on the supporting tube J) is pushed up the rod to a height corresponding to the total compression. This sleeve stays unmoved when the cheese recovers and, after reading $\epsilon_{\text{plastic}}$, if the grub screw is loosened, the compression body slides down until H comes back into contact with the supporting tube J. The vernier now reads the maximum deformation, ϵ_{total}, which was produced under the pressure. The elastic deformation is given by $\epsilon_{\text{elastic}} = \epsilon_{\text{total}} - \epsilon_{\text{plastic}}$.

The value of $\epsilon_{\text{plastic}}$ naturally depends on the time of application of the stress, but experiment has shown that reproducible results can be obtained without difficulty if the pressure is applied for an estimated half-second.

At the time of writing enough has been done to be confident that the method works easily and with reasonable reproducibility, but there are not yet enough data to justify their publication or to draw any conclusions from them. The authors are indebted to Mr H. S. Hall for the construction of the apparatus.

About forty Cheddar cheese were made at factory B in connexion with another experiment (4) and, although unusual treatments were given in some

cases, almost all the cheese were judged to be of normal type when ripened. For the majority of these, pitching numbers had been obtained. The cheese were judged by five expert graders, who were asked to give separate judgements of body, texture and smell. As already stated, expert graders find it

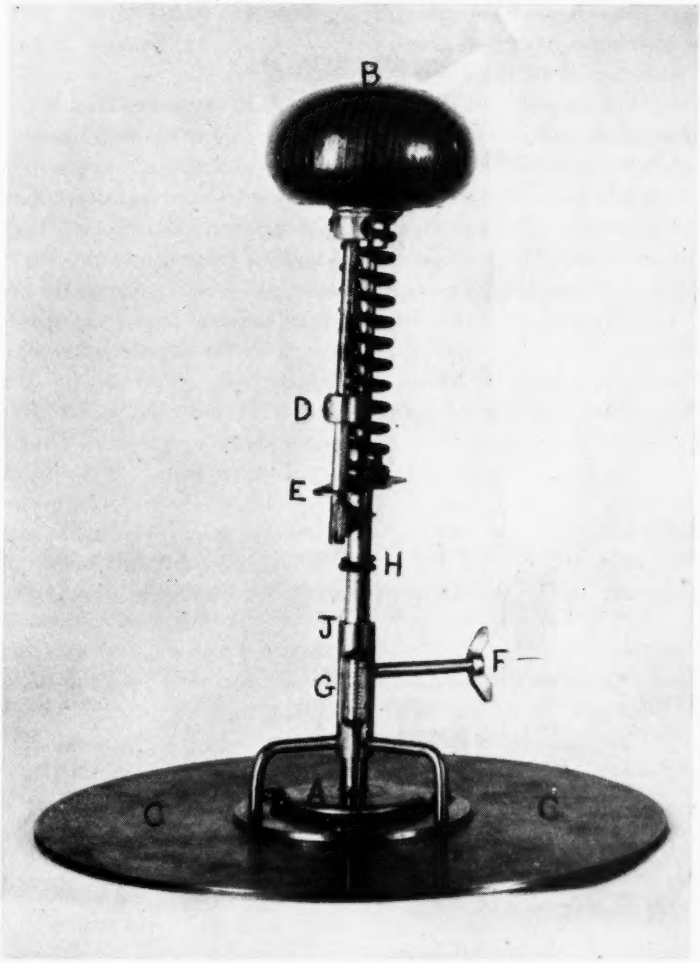

Fig. 3.

extremely difficult to separate quality of cheese into components. As the *Lancet* puts it(5): "It is rather like asking a doctor to base his opinion of the patient on the result of using a stethoscope only." One of us (G. W. S. B.) gave a subjective judgement of firmness alone before making the objective

measurement. In order to calculate correlation coefficients, the judgements were assessed numerically as follows:

Very firm	Firm	Rather firm	Slightly firm	Very slightly firm	Normal
+5	+4	+3	+2	+1	0

Very slightly soft	Slightly soft	Rather soft	Soft	Very soft
−1	−2	−3	−4	−5

The correlation coefficient was 0·73 ($P = \ll 0{\cdot}01$).

The judgements of the experts were difficult to assess numerically, but an attempt was made to group them into five categories. These judgements did not correlate significantly with the S values, nor did the judgements of different experts agree except in the case of two of them, who compared notes during their examination. On the other hand it must be remembered that the experimenter, in judging firmness, knew that his judgement was to be correlated with S, which is only one way of measuring "body", whereas the experts had no such indication of the purpose of the tests. It seems impossible that the objective test could have been influenced by the experimenter, who had just given a subjective judgement.

The correlation coefficient was calculated between the W/h values of thirty-five cheese and the S values obtained when the cheese were ripened, but proved to be insignificant. It has already been noted that in factory B the variability of pitching point is very slight, and this is probably the reason. In a special experiment, three cheese were made as similarly as possible except that the scalding was continued for different periods and to different temperatures. After ripening, the S values were determined. The results are given in Table 5.

Table 5

Cheese	Time of scald min.	Maximum temperature	W/h	Moisture	S (after 61 days) (mean of five)
1	59	99	20·3	59·0	7·78
2	92	102	18·9	54·5	8·04
3	103	106	18·6	49·8	8·44

It does not, of course, follow that the best cheese has the highest value of S. In fact, in this experiment, the softest cheese was judged by two of the experts as the best of the three. A wider experiment to test the same point is already in progress.

Conclusions

The quality of cheese, and especially the keeping quality, is much affected by the consistency of the curd at pitching. However skilled the individual cheesemaker may be, it is clearly undesirable, especially in war time, that the fixing of the pitching point should be dependent on the subjective judgements of a single individual. An unexpected demand for long- or short-keeping quality cheese or for cheese to suit exceptional climatic conditions could be

much more effectively met by a standardized technique referring the condition of the curd at pitching to an objective scale. This does not mean that all factories should pitch at the same point on the scale. Local conditions, type of milk and market, pasteurization, etc. must all be taken into account. What is important is that the best technique for each factory be consciously chosen and that further studies be made to establish the laws relating the now measurable pitching consistency to the body and ripening properties of the finished cheese.

It is a mistake to suppose that quality of foodstuffs becomes unimportant in times of national crisis. On the contrary, when quantity may have to be reduced, quality becomes increasingly important from the point of view of nutrition. Quality cannot be adequately assessed in terms of chemical composition alone. In a state of siege, people have been known to eat shoe leather, but the high protein content of this material is no criterion of its food value.

Here again, expert subjective judgements are not enough. It appears probable that the food value of cheese depends to some extent on certain of its physical properties. These must be measured and controlled by means of a scientifically planned dairy technique. The question of palatability must not be neglected, since this affects digestion, and there is no doubt that the physical properties of cheese are closely linked with the types of micro-organisms developed therein (3).

Summary

1. The pitching consistency of some 470 cheese has been determined at one factory and the data compared with the subjective judgements of a skilled cheesemaker. The smallest difference in consistency which the expert can distinguish with a reasonable degree of accuracy (threshold) is compared with those for purely elastic and viscous bodies, and it is concluded that the consistency at pitching is judged mainly from the elastic properties of the curd. The good agreement between these thresholds substantiates the hypothesis that our test for pitching consistency is closely related to the requirements of the practical cheesemaker.

2. The connexion believed to exist between firmness of curd at pitching and acidity was not substantiated, in the case of Cheddar, by a statistical examination of the available data.

3. The most usual values (medians) for pitching consistency are compared for four different factories, and it is shown that differences in technique may be associated with the same pitching consistency and produce very similar cheese, but that in other cases good cheese may be produced from very different pitching consistencies. The important thing is that the pitching consistency should be known and controlled so as to be as constant as possible under given conditions.

4. A technique is described for measuring the force required to thrust a skewer into cheese. This force (S) is highly correlated with "body" judged

subjectively by an experimenter who was aware of the fact that such a correlation was to be tried. Judgements of "body" made by expert cheese graders were not so correlated, but the interpretation of their judgements was inadequate, so that no definite conclusion can be drawn.

ACKNOWLEDGEMENTS

Our best thanks are due to a number of cheesemakers and others who have co-operated in this work and especially to Miss V. E. Cheke and the staff of the British Dairy Institute, Reading; to the director and staff of the Somerset Farm Institute, Cannington, particularly Miss M. Taylor and Miss E. M. Monie of that Institute; to Messrs Aplin and Barrett, Yeovil, for permission to publish data from their factories, and to Dr E. L. Crossley for his help and co-operation at Yeovil and Frome. We are also indebted to the late Mr F. J. Dix, from whom we received much help both in the construction of apparatus and in the statistical treatment of results, and to Mr J. Cotton, who has designed a modification of our testing instrument more suitable for industrial use.

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(*MS. received for publication* 12 *October* 1940)

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274. PRELIMINARY EXPERIMENTS ON THE VAPOUR PRESSURE OF DAIRY PRODUCTS

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(With 3 Figures)

General introduction

Two preliminary series of experiments have been performed to investigate the usefulness of vapour pressure measurements in the study of dairy products. These can be grouped as (*a*) experiments on the vapour pressure of different types of cheese and its relation to various factors such as fat content, salt content, the connexion between bound water and the process of ripening, and (*b*) experiments on the vapour pressure of milk, in order to find an alternative method of determining the osmotic pressure and hence the amount of watering.

There are two main methods of measuring the vapour pressure of a substance—static and dynamic. The former measures the pressure of the saturated vapour of the substance in a vacuum, either directly with a mercury column, or differentially, against a substance of known vapour pressure with a differential manometer. This method, as developed by Lovelace, Frazer and co-workers (1, 2, 3, 4) and Chandler (5), is the more accurate, but is too intricate for the present purpose and requires too much time. The dynamic, measurement may be derived from the weight of vapour carried over by a given amount of gas bubbled through the substance (6, 7, 8), a method obviously unsuitable for cheese; or differentially by allowing the unknown substance to distil in a closed space with a substance of known vapour pressure. This latter method is the one adopted in the present experiments. The earliest mention of it seems to be by Barger (9), who arranged alternate drops of solutions of known and unknown vapour pressures in a fine tube, and measured the change in length of the drops with time, as the drops with the higher vapour pressure distilled into those with the lower. The vapour pressure was found by repeating the experiment with several solutions of known vapour pressure and interpolating on the length-change/known vapour pressure curve at the point where the length-change is zero. This method was extended by Bousfield & Bousfield (10), who called it the "isopiestic" method. Criticisms were made by Sinclair (11), who improved the method. Other techniques include the dew-point method of Hepburn (12) and Hill (13, 14), which was also developed by others (15, 16). These appear suitable for measuring the vapour pressure of milk.

[1] Mr Dix died before the writing of this paper had been completed.

Experiments on cheese

Introduction

The earlier measurements were made to develop a technique suitable for simple and accurate determinations of vapour pressure to be used as a measure of bound water. The purpose was to investigate the connexion between the growth of micro-organisms necessary for the blueing of Stilton cheese and the condition and quantity of moisture available.

The conception of the term "bound" water seems to vary. Pyenson and Dahle (17, 18, 19) have determined the bound water in liquid dairy products by the cryoscopic method. They define bound water as "water which will not dissolve sucrose", and as "the term applied to water held on the surface of colloidal particles, water of hydration, etc." A criticism of measurements of "bound" water by the cryoscopic method is given by Kistler (20). By "bound" water in this paper is meant that water which is so held that the micro-organisms cannot utilize it. This includes water held by chemical bonds, such as water of hydration, water bound by colloidal particles and water which is bound by materials dissolved in it. This is somewhat analogous to Schofield's (21, 22) conception of pF. He pointed out that the suction pressure required to extract the water from various soils could vary from 1 or 2 cm. of water, up to that exerted by a column of water higher than Mount Everest. He therefore proposed a logarithmic pF scale similar to the pH scale only differing in sign. He related the pF of a material to the vapour pressure exerted by the moisture held in that material by the equation:

$$p\mathrm{F} = 6{\cdot}51 + \log_{10}(2 - \log_{10} h) \text{ for water at } 20^\circ \text{ C.},$$

where h is the relative humidity.

The micro-organism will have to obtain its moisture against this suction, so it seems reasonable to consider the vapour pressure as a measure of the availability of the water to the organism, i.e. of the "bound" water.

Very little work has been published on the osmotic pressure or bound water of cheese, except that Sommer (23) found that the freezing-point of cheese decreased with age, while the moisture content remained almost constant, showing that the amount of "bound" water increases with age.

Experimental

In our early experiments cheese and salt solution were placed in watch glasses, side by side in a closed Petri dish, and were allowed to distil at a fairly constant temperature. In one glass was placed grated cheese and, in the other, one of a series of concentrated solutions of different salts with relative humidities ranging from 0·81 to 0·98. The time usually allowed for the solutions to come to equilibrium was 48 hr. The weight-changes were plotted against the relative humidities and the relative humidity of the cheese found by interpolating for zero weight-change. This avoids any assumption as to the type of equilibrium

reached. Later, solutions of common salt of various concentrations were used instead of the saturated solutions of different salts. A table was obtained showing the vapour pressure of these solutions, for which we are indebted to Dr Henry and Dr Withers of the Shirley Institute. The remainder of the experiments were performed using these solutions. We are indebted to Dr R. K. Schofield for the following improved technique. A large number of airtight tobacco tins of diameter about 4·5 in. were fitted with a simple wire clip to hold a pad of cotton wool, about 2 in. square, against the lower side of the lid. The cheese was spread thinly on the bottom of the weighed tin, which was then reweighed as quickly as possible to avoid evaporation. A pad of cotton wool was then soaked in the required salt solution and was lightly pressed out to prevent dripping. The pad was quickly clipped against the lid and the tin tightly closed. After 48 hr. at a constant temperature, approximately 60° F., the lid was removed and each tin and cheese weighed immediately. The change in weight per g. of cheese was then plotted against the salt concentration, and that concentration corresponding to no change in weight was obtained by interpolation. In the later experiments on blueing of Stilton cheese corrections for salt content and moisture content were introduced.

Assuming as a first approximation that the sodium chloride present in the cheese was simply dissolved in the water also present, the vapour pressure of this solution was subtracted from the total vapour pressure. It is realized that this cannot give a complete correction for salt effect, but a vapour pressure corrected in this way was worked out in each case to see whether such a correction would improve the correlation with blueness or the reverse.

The curves plotting vapour pressure against moisture content were markedly curvilinear. By plotting the reciprocal of moisture content a practically linear curve was obtained.

The correction for moisture content involved a difference in the experimental technique. The vapour pressure of portions of the cheese was measured as above, and the cheese then allowed to dry off for a time, after which the vapour pressure and moisture content were again measured. These measurements were repeated in some experiments more than once. The vapour pressure was then interpolated for an arbitrary convenient moisture content (28·6).

The tins were left for 48 hr. although the equilibrium was not then fully attained, but the interpolation method does not require that it should be.

Results

Effect of air-drying and spraying

A Cheddar cheese was divided into two halves and the same experiment was done on grated portions of each half. The cheese was air-dried, moisture contents and vapour pressures being determined at intervals. It was then artificially moistened by spraying, the vapour pressure being determined at various moisture contents.

The results of this experiment are shown in Fig. 1, in which it is seen that there is no hysteresis shown in vapour pressure as moisture is given out and taken up. This is in contradistinction to the effect for soil discovered by Schofield (21) who, discussing loam, states that there "is no doubt, however, that over the central portion of the moisture range (that of most importance in the field) the suction this soil can exert in taking up moisture is only about one-quarter that required to withdraw water from it at the same moisture content" (see (21), Fig. 1, p. 40). Schofield points out, however (private communication), that the behaviour of cheese is more likely to approximate to that of a material such as gelatine than to that of soil. Provided that the range of pF is not too great, gelatine shows no hysteresis.

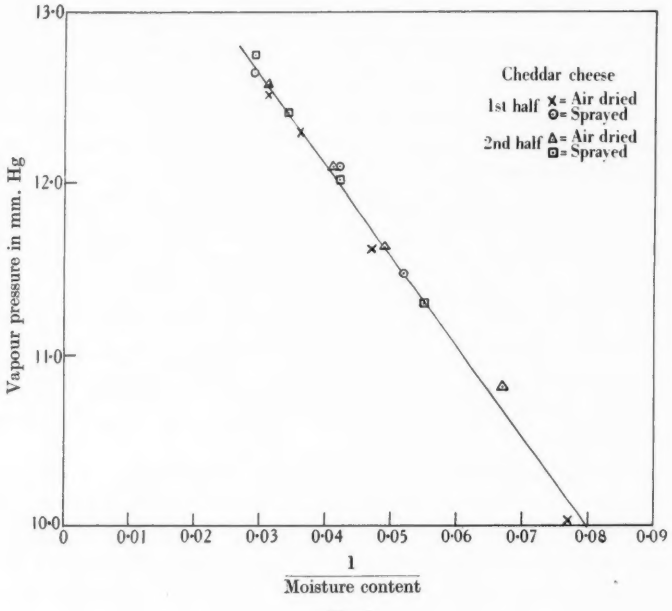

Fig. 1.

(b) Connexion between vapour pressure and moisture content for different types of cheese

The moisture contents of the cheese were reduced by drying, both these and the vapour pressures being measured at various stages.

The results are shown in Fig. 2. The vapour pressure of pure water is shown by a horizontal line. It will be noticed that there appears to be an intercept on this corresponding to a moisture content of 57 %. It is probable that this may be partly due to a slight leakage from the tins. This might give rise to an effect of this order, and it may be more correct to assume the leakage as constant and to consider all the vapour pressure readings to be about 0·6 mm.

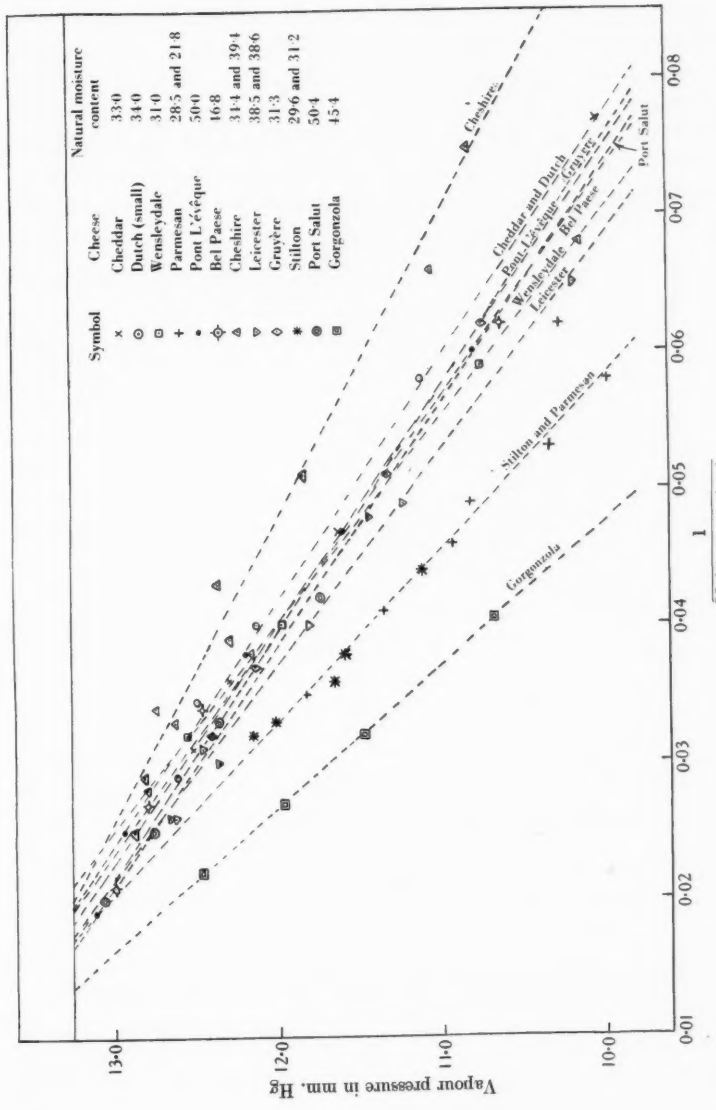
Natural moisture content
Symbol Cheese
× Cheddar 33·0
⊙ Dutch (small) 34·0
□ Wensleydale 31·0
+ Parmesan 28·5 and 21·8
• Pont L'évêque 50·0
◇ Bel Paese 16·8
△ Cheshire 31·4 and 39·4
▽ Leicester 38·5 and 38·6
◇ Gruyère 31·3
✱ Stilton 29·6 and 31·2
● Port Salut 50·4
■ Gorgonzola 45·4
Cheshire
Cheddar and Dutch
Pont-L'évêque
Gruyère
Wensleydale
Bel Paese
Leicester
Port Salut
Stilton and Parmesan
Gorgonzola
Vapour pressure in mm. Hg
13·0
12·0
11·0
10·0
0·01
0·02
0·03
0·04
0·05
0·06
0·07
0·08
1/Moisture content

Fig. 2.

Hg too great. There is, however, no reason to suspect the relative positions of the points. The slopes would still be practically unaffected.

(*c*) *Vapour pressure of cheese made with varying salt contents*

Through the courtesy of the British Dairy Institute, five Cheddar cheese were made containing progressively increasing amounts of salt. From the vapour pressure curves shown in Fig. 3 it is apparent that, as would be expected, the effect of adding salt is to decrease the vapour pressure. Although the effect of salt on the slope of the vapour pressure curves is considerable, it is not sufficient to account for all the differences shown between the different types of cheese (Fig. 2). Some preliminary experiments were also done on varying fat contents, but the data were inadequate to draw definite conclusions.

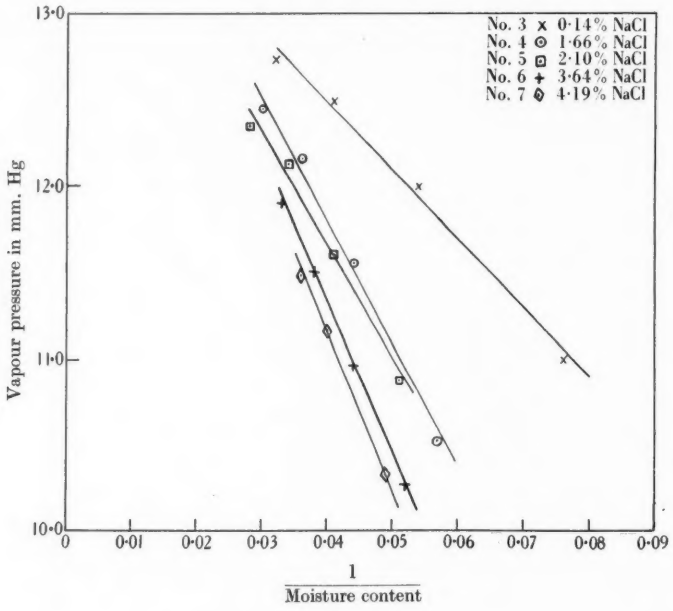

Fig. 3.

(*d*) *The connexion between the rate of blueing of Stilton cheese and the amount of the bound water present*

The vapour pressures of a set of Stilton cheese were obtained at a fixed arbitrary moisture content by interpolation and were calculated both corrected and uncorrected for salt content.

Although it appeared that some connexion existed between these vapour pressures and the extent of blueing of the cheese, the whole question is complicated by the fact that the amount of blueing depends on the amount of moisture that has been available for the organism during the whole period of

ripening. Although the vapour pressure is corrected for differences in moisture content at the time when it is measured, the blueness figure, which is estimated subjectively, cannot be corrected in the same way. What is required is an experiment in which the vapour pressures and moistures are measured regularly during the whole course of ripening. A more objective method for estimating blueness is also needed. Further, it is clear that some at present unknown combination of vapour pressure and moisture content is required for correlating with "blueness". It is intended that this matter shall be further investigated.

Experiments on milk

These experiments were interrupted by Mr Dix's death at a stage which makes it impossible to give quantitative data. It is hoped to extend this work so as to develop a technique for the rapid determination of watering in milk and also, by applying the more refined thermocouple technique of Hill and others (13, 14, 15, 16), to make a fundamental study of the osmotic pressure of milk in relation to other body fluids.

By means of a technique essentially similar to that described for cheese, curves were obtained relating vapour pressure to amount of added water for artificially diluted milk. Although the technique has not yet been fully developed, it was possible even at this early stage to detect, with considerable assurance, the addition of about 2 % added water.

We are indebted to Mr D. Maurice, who carried out many of these tests and also made a preliminary study of the potentialities of Barger's (9) method which also seems worthy of further study.

Summary

1. Methods for measuring the vapour pressure of cheese and milk are discussed, and vapour pressure/moisture curves are given for a number of different varieties of cheese. The progressive artificial damping and drying of cheese produces no appreciable hysteresis in these curves.

2. The vapour pressure curve is much influenced by the amount of salt in the cheese, but differences between varieties cannot be accounted for entirely in terms of differences in NaCl content.

3. A preliminary experiment on the relationship between vapour pressure of Stilton cheese and amount of blueing indicated that such a relationship does in fact exist but that a much larger experiment is required before the connexion is fully understood.

4. Preliminary experiments on the measurement of the vapour pressure of milk, although interrupted at rather an early stage, showed that, even so, additions of 2–3 % of water in milk can be satisfactorily detected.

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(*MS. received for publication* 12 *October* 1940)

637.333:576.809.6

275. STARTER CULTURES FOR CHEESE MANUFACTURE

FURTHER ATTEMPTS TO ELIMINATE FAILURES DUE TO BACTERIOPHAGE

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Cheese-starter cultures frequently suffer failure owing to the action of bacteriophage in suddenly causing lysis of the streptococci. In a previous paper(1) it was suggested that the bacteria themselves could give rise to the phage when they were existing under the somewhat unfavourable conditions obtaining immediately after their transfer to a fresh batch of milk. Hence it was recommended that the lag period in the development of a culture should be reduced as far as possible and that rapid growth should be encouraged by the use of

(*a*) Massive inoculation.

(*b*) Relatively high incubation temperature (75° F.).

(*c*) Milk freshly heated and cooled—thus containing a minimum of dissolved air.

The application of these principles in commercial practice appeared to give very good results, but there still occurred instances of failure which we ascribed to infection of the starter with phage through the use of a faulty technique by the operator. Eventually, however, a failure occurred where we were reasonably certain that the technique of subculturing was not at fault. Consequently we were driven back to the suggestion (often advanced before) that some abnormality in the milk used caused the streptococci to produce the destructive phage. A re-examination of this matter of the possible influence of milk composition on starter maintenance gave negative results. Milk from various commercial factories, milk from cows suffering from mastitis (both acute and chronic), and colostral milk, all proved satisfactory media at the Institute for the continued propagation of strains of streptococci used as starters. For some months no progress was made. In the commercial factory (Bell Block Dairy Company, New Plymouth) where the unexplained failures had occurred there was recurrent trouble whatever culture was in use.

Environmental factor disclosed

Finally it was suggested that the possible influence of the milk in the Bell Block factory might be eliminated by the expedient of preparing the starter in reconstituted milk made from skim-milk powder. The powder was prepared

in another district and from very large bulks of milk. It seemed unlikely, therefore, that it would have the same postulated abnormality as the milk at Bell Block. Several hundredweights of powder prepared at different times were obtained and each was divided into two portions. One portion of each was taken to Bell Block and the other was retained at the Institute. The same procedure was then followed at both places. Each day 10 gallons of reconstituted milk was made by dissolving 10 lb. of powder in 10 gallons of water. The milk was "sterilized" in the manner commonly practised in the preparation of bulk starter and it was inoculated with a single strain culture (HP). In this experiment a mother culture was not kept; the starter was transferred from can to can daily by means of a sterilized dipper. Culture HP treated in this way at the Institute gave no sign of failure and still showed its normal activity at the end of a month. At Bell Block, on the other hand, a failure occurred on the third day, the inoculated milk being still quite liquid after 18 hr. incubation and a strong concentration of phage was present. A new start was made with a fresh mother culture of HP. Failure again occurred on the third day while a third trial gave a failure in 2 days. This experiment made it quite evident that some factor in the environment at Bell Block was the cause of the failure. It was no longer necessary to assume an abnormality in the milk. It seemed almost certain that the environmental factor must be air-borne bacteriophage and experiment soon confirmed the suspicion. It was thus evident that our previous conclusions as to the origin of phage were wrong, at any rate in some instances.

Demonstration of air-borne phage

Three methods were used to demonstrate the presence of phage in the atmosphere at Bell Block and later in other factories.

(*a*) *Aspiration of air.* Air was aspirated through sterile water in test tubes fitted with bungs carrying suitably bent glass tubes. About a litre of air was drawn through 10 ml. of sterile water. Air was sampled in several parts of the Bell Block factory in this manner. On return to the Institute the water in the various tubes was tested for presence of phage (HP phage in this instance) in the following way. Five ml. of water from each tube were added aseptically to a 10 ml. tube of sterilized skim milk; the milk tubes were inoculated with culture HP. After incubation at 22° C. overnight the tubes were examined. Those which failed to clot were assumed to contain phage, an assumption which was checked on numerous occasions and found to be correct. The clotted cultures were subcultured into tubes of sterilized skim milk at 22° C. and this procedure was repeated for 3 or 4 days. Some samples of water caused a failure to clot after the second or third subculture, and it was invariably found that the failure to clot coincided with the development of a strong concentration of phage.

In a few instances the inlet tubes in the pieces of apparatus described above were plugged with cotton wool. It was found that the wool always retained

the phage when a litre of infected air was drawn through the apparatus; the water in the tube contained no phage while the wool plug, added to a tube of sterilized skim milk inoculated with culture HP, caused a failure to clot within one or two subcultures.

(*b*) *Exposure of sterilized skim milk.* A simple method for detection of phage in the atmosphere and one which was later adopted as a standard, consisted in the exposure of 10 ml. of sterilized skim milk in a sterile Petri dish on the selected site for 10 min. After the exposure the milk was transferred by means of a sterile pipette to a sterile plugged tube and on return to the laboratory the milk in the tube was inoculated with the appropriate strain of *Str. cremoris*. The subsequent procedure was the same as in the first method.

(*c*) *Exposure of inoculated agar surface.* The most direct method for demonstration of phage was the exposure of an agar plate which had been spread immediately beforehand with a culture of the appropriate streptococcus. After a 10 min. exposure the plate was incubated at 30° C. for 24 hr. Examination of the confluent growth for clear plaques was then made. This method was a very direct proof of the presence of phage where the air-borne concentration was high; thousands of tiny plaques were often observed on plates after exposure in dairy factories. The first two methods were, however, much more delicate, since a few plaques on a plate might easily escape detection.

It is perhaps as well to re-emphasize here the fact that phage can be demonstrated by the above methods only when pure strains of *Str. cremoris* are used as substrate cultures. Phage cannot be detected by the use of a mixed culture either in liquid or on solid media. The relative specificity of phages makes it unlikely that all the streptococci in a mixed culture will be attacked; hence with a mixture of streptococci a clear-cut result is impossible.

A survey made at several factories by the use of the above methods was most illuminating. The following significant findings were made:

(1) Phage for the particular culture in use as a starter was almost always present in the whey in the cheese vats. This had been noted previously (1).

(2) As was to be expected, phage was invariably present on dust or earth collected under the whey tanks outside the factory.

(3) Phage was regularly present in the air in the main factory buildings.

(4) Dust from beams and ledges in the factory contained phages for cultures which had lately been used in the factory.

(5) The strongest concentration of air-borne phage was found in the neighbourhood of the whey separator.

The whey separator proved on further investigation to be the centre from which most of the air-borne phage emanated. All cheese whey in New Zealand dairy factories is passed through a centrifugal separator for recovery of the butterfat. Apparently the stream of separated whey emerging from the rapidly revolving bowl and impinging on the stationary cover is atomized to some extent, with the result that the separator emits an invisible "mist" consisting of fine particles of whey usually containing a high concentration of phage.

Tests showed that the whey "mist" spread in ever-widening circles throughout the period of operation of the separator and that the finely divided particles remained in suspension in the atmosphere for several hours. It soon became apparent that this form of air-borne phage was responsible for most of the hitherto unexplained cases of starter failure.

Air-borne phage in relation to previous findings

How could the discovery of the prevalence of air-borne phage in commercial factories be reconciled with our previous findings?

(1) Firstly, there was the fact that the adoption of the system of massive inoculation in the Institute factory had eliminated starter trouble there. Three factors appeared to be concerned:

(*a*) The cheese whey was not run through a separator at the Institute. Hence although traces of air-borne phage were occasionally present in the experimental factory, there was not the high daily concentration due to the atomization of whey in a separator.

(*b*) The mother culture was maintained in the laboratory out of range of even the small amount of phage present in the air in the factory.

(*c*) Massive inoculation of the bulk starter in the factory was capable of over-riding the slight occasional infection with air-borne phage. In experiments on the addition of phage to cultures described in a previous paper (1) we found "that the only specific effect of heavy inoculation was to preserve some of the cultures from phage action for one generation longer than was the case with light inoculation". The import of this observation was not realized at the time. Emphasis was laid on the eventual failure of the cultures even when massive inoculation was practised rather than on survival for one extra propagation. Where the mother culture is concerned one extra propagation is certainly not of much moment, but with the bulk starter it is a different matter. The bulk starter is used only for one further propagation (i.e. in the cheese vat), and the fact that a massive inoculation may preserve it from failure for one extra generation in the presence of contaminant phage means all the difference between success and failure in the cheesemaking process. It seems to us now that the occasional failures in the Institute experimental factory in the past must have been due to occasional slight phage infection of the bulk starter from the air, and that the infection was so slight that a more massive inoculation was sufficient to preserve the culture from failure until the cheesemaking process was finished.

(2) All the results reported on the effect of aeration and "ageing" of sterilized milk and the effect of size of inoculum needed to be viewed in a new light. It was now evident that the process of aeration implies an exposure to air-borne infection. Further, aeration, "ageing" and light inoculation severally or in combination tend to prolong the lag period which occurs before the streptococci start to multiply rapidly. This prolongation seems to make it more

probable that contaminant phage will develop sufficiently to lyse the bacteria before they can clot the milk. Thus whereas previously it was postulated that these factors tending to delay the growth of the streptococci in milk would encourage the bacteria to give rise to phage, it was now possible to explain the results on the hypothesis that *provided contaminant phage was present in the milk* (due usually to air-borne infection) the factors mentioned above would, by prolonging the lag period, enable phage to develop and lyse the bacteria much earlier than would otherwise be the case.

(3) The apparent "periodic sensitivity" of streptococcal cultures to spontaneous phage failure (2) in aerated milk was evidently a fallacy. The spasmodic occurrence of failures was due not to variations in the condition of the cultures but to variations in the degree of contamination of the milk with air-borne phage during the aeration process. It is still not clear why air-borne phage should be more prevalent in the laboratory at one time than at another. Possibly weather conditions determine whether or not the dust particles and droplets settle rapidly or remain for a long time in suspension. It is, however, noticeable that, since the institution of more stringent precautions in the washing and sterilizing of phage-infected glassware, increased difficulty has been experienced in obtaining "spontaneous" phage appearance in milk aerated in the laboratory in recent years.

(4) The sometimes quite long periods of successful maintenance of starters in commercial factories appeared rather puzzling. Up to the present it has not been possible to clarify this point completely, but on several occasions some possibly significant factors have been observed. Air-borne phage is necessarily carried about by air currents and draughts. Commercial factories vary considerably in their lay-out and in the disposition of the various pieces of equipment. Depending on the lay-out of the factory and on the prevailing wind, the phage-laden air around whey tanks and separator may be carried towards or away from the starter cultures. Instances have been observed where it seemed almost certain that a period of successful maintenance of the starter was essentially due to a "protection" of the culture by air currents. In these cases a change in wind direction was followed by starter failure.

Apart from the factor of air currents it is possible that differences in milk composition and in the technique of cheese manufacture may result in a variation in the concentration of phage in the cheese whey in different factories and in the one factory from time to time. We have no evidence on this point at present.

Reviewing the position as a whole it is apparent that the hypothesis of spontaneous phage development under the influence of aeration and of factors delaying bacterial growth must be abandoned. It seems more likely that air-borne infection occurs sufficiently often to account for most of the phenomena observed.

Origin of bacteriophage

The recognition of the prevalence of air-borne phage invalidates most of the evidence which was previously adduced in favour of the hypothesis that the bacteria themselves give rise to phage. Even the occurrence of a new phage on a resistant culture in its first propagation in a dairy factory (reported by the present authors (3)) has lost much of its weight as evidence of a bacterial origin for phage, since the phages have been shown to be not so strictly specific as at first they appeared to be. We have encountered one phage which attacks four distinct strains of *Str. cremoris*. It is possible, therefore, in the instance just mentioned, that a phage capable of attacking the resistant culture was present in the air of the factory as a result of the previous use in the cheese vat of a mixed culture which happened to contain a streptococcal strain susceptible to attack by a phage capable of acting on our resistant culture. This phage may have been developed daily in the cheese whey for some considerable time and have been distributed by the action of the separator.

The occasional occurrence of phage in the whey in vitality tests in spite of all possible precautions against infection is not convincing as evidence of a bacterial origin for phage. It would become so only if the phage could be caused to occur at will as a result of some specific variation in technique not involving any risk of contamination from extraneous sources. Such a variation in technique has not been found up to the present.

Mode of development and spread of phage in factories

If we assume for the moment that phage does not originate in the starter cultures the evidence at present available suggests the following sequence of events in commercial practice.

A culture of a single strain of *Str. cremoris* behaves quite normally in a commercial factory until a particle of a phage capable of attacking it happens to fall into the cheese vat. The vat may be regarded as a huge culture of *Str. cremoris* presenting for several hours a large surface open to contamination by particulate matter in the atmosphere. When a particle of the appropriate phage falls into the vat it multiplies with the streptococci, but not to the extent necessary to cause lysis. The whey on removal from the vat thus contains some phage which is distributed in the air by the atomizing action of the separator. The phage falls into the vat in increased quantity next day, with a resultant increase in its concentration in the whey. Soon there is regularly a high concentration in the cheese whey, although the starter still develops the required acidity in the vat because the cheesemaking process is finished before the concentration of phage necessary to cause lysis of the bacteria has been reached. In our experience the streptococci never suffer lysis in the vat as a result of contamination of the vat and utensils from the air or from stale whey, although a failure would be possible if enough stale whey were ever allowed to be present. Failures always seem to occur as a

result of contamination of either the mother culture or bulk culture. This probably means that the extent of contamination is such that the phage has to develop for one propagation before it attains the requisite concentration to cause lysis in the next propagation. Alternatively the phage may not be able to attack the streptococci so readily in cheese curd as in liquid milk. Be that as it may, when a failure in a cheese vat occurs we invariably find that the starter added to the vat contains demonstrable phage and on the other hand that most normally running cheese vats contain phage although none is present in the starter.

The sequence of events outlined readily accounts for the presence of air-borne phage in the factory building and this in turn accounts for failures which occur when an attempt is made to subculture starters inside the factory. Instances have been observed where the removal of the wool plug from a flask for 10 sec. was sufficient to admit enough air-borne phage to cause failure of a culture within 24 hr. A change to another starter culture which has not been used before restarts the cycle. There is no trouble for a period which varies from a few days to a few weeks. Then phage for the fresh culture appears in the whey and is spread by the separator until the culture suffers failure due to air-borne phage.

ATTEMPTS TO ELIMINATE PHAGE FAILURES IN COMMERCIAL PRACTICE

Three methods for elimination of starter failures due to phage are at present undergoing trial in commercial factories.

(*a*) Destruction of air-borne phage by disinfectants in spray form.

(*b*) Protection of cultures in the factory from air-borne phage.

(*c*) Preparation of the starter in a separate building designed to exclude phage-laden air.

It is too early as yet to reach a definite conclusion on the relative effectiveness of the different methods, but over the course of a dairying season several clear indications have been obtained.

The destruction of air-borne phage by spray treatment is exceedingly difficult. The disinfectant must be distributed in a very fine state if it is to be effective at all, and since, in the main factory building, reinfection is continuous it is not possible to do more than protect the cultures for a short time during the process of subculture.

Protection of the bulk culture is possible to some extent by making air-tight the lids of the cans in which the culture is prepared and providing a small opening plugged with cotton wool through which the milk can be inoculated. Thus the air-borne phage which gets access to the bulk starter can be limited to an amount below that necessary to give trouble before the starter has done its work in the cheese vat.

The provision of a separate building at a distance from the factory and the provision of means for excluding all air-borne phage seems likely to be the

only completely satisfactory means for eliminating trouble. Whereas the bulk starter may be prepared under conditions which permit a slight contamination with phage, the mother culture must be protected completely, since any trace of phage in it ultimately destroys the culture. A separate building seems to be the only means for ensuring this at present. At Bell Block factory this system was given a trial. A special building consisting of two rooms was erected. In one room the bulk starter was prepared, while the other inner room was used for the subculture and incubation of mother cultures. Over a season of 9 months the trial was completely successful. The starters were maintained just as successfully as at the Institute and the cheesemaking process was reduced to a regular routine. Similar but less comprehensive trials in other factories tended to confirm the results and there were clear indications that any system which helped to protect the starters from air-borne phage gave relief from phage failures. Discovery of the best methods for putting the basic principles into practice must await the results of continued trials under commercial conditions.

SUMMARY

Bacteriophages for lactic streptococci were found to occur in the atmosphere of commercial cheese factories. Finely divided particles of whey emitted from the whey separator appeared to be the main vehicle for the air-borne phage although whey-contaminated dust probably also played a part. The concentration of air-borne phage was sometimes so great that, with the use of a normal bacteriological aseptic technique, it was impossible to prevent infection of the starter cultures for more than a few propagations. Protection of the starter from air-borne phage eliminated phage failures.

The question of the origin of bacteriophage is discussed in the light of the recognition of the prevalence of air-borne phage. The occurrence of air-borne infection could account for most of the instances of starter failures which were previously thought to be due to spontaneous phage development within cultures.

The authors' thanks are due to Prof. W. Riddet and Drs F. H. McDowall and I. R. Sherwood for their constant interest and helpful criticism; also to the Manager of the Bell Block Dairy Company, Mr J. Thompson, without whose co-operation the trials on a commercial scale would not have been possible.

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(*MS. received for publication* 5 *December* 1940)

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276. THE DRYING OF CHEESE WHEY AND OF ACID CASEIN WHEY BY THE ROLLER PROCESS

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(With 2 Plates)

For certain purposes it is necessary to produce roller-dried whey from cheese whey which has been brought as near as possible to the neutral point. The first part of this paper deals with problems involved in the drying of such neutralized cheese whey. The second part deals with the parallel problem of the neutralization and drying of whey which is formed as a by-product in the manufacture of acid casein.

1. The drying of neutralized cheese whey

Whey which has been freshly drawn from the cheese vats usually has an acidity of 0·15–0·20 % expressed as lactic acid.[1] In large creameries considerable volumes of whey may, however, be drawn off from the vats many hours before it can be dried. Acid development during such storage will be rapid unless special precautions are taken, acidities up to 0·30 and even 0·40 % being not uncommonly encountered in practice. Methods of preventing or at least of minimizing such excess acid development (such as refrigeration or pasteurization) are, of course, well recognized, but local circumstances may exist which preclude their adoption. Moreover, even if excess acid development is prevented, there still remains the question of how best to neutralize the acidity of the original whey.

The chief difficulty in this connexion is to neutralize the whey without affecting its drying properties on the rollers. Normally, with whey having an acidity between 0·15 and 0·25 %, the scraper knife collects a molten mass, which quickly forms a dry skin, breaks up and drops off in the form of small irregular pieces, somewhat resembling popcorn in shape and size [see Plate 1 (*a*)] Preliminary experiments carried out on roller-drying machines at two

[1] The custom of the dairy industry of describing the acidity of milk or whey in terms of percentage lactic acid is misleading. The figure so obtained is actually a measure of the buffer action of the salts, together with an acidity derived only partly from lactic acid. In the whey resulting from acid casein manufacture the use of such nomenclature is manifestly absurd, since in such a case the acidity is almost entirely due to the precipitant, i.e. either hydrochloric, sulphuric or acetic acid. However, the trade practice has for convenience been retained throughout this paper. The figures given have been obtained by dividing by ten the titre obtained when 10 ml. of whey were titrated against $N/9$ sodium hydroxide solution, using phenol phthalein as indicator.

creameries in Scotland showed, however, that if the whey was previously neutralized with sodium bicarbonate it behaved in a very different manner; the molten material collecting at the knife failed to form a dry outer coat and remained as a continuous liquid strip which revolved continuously against the knife and consequently became abnormally brown and caramelized. Moreover, the evolution of large volumes of CO_2 caused such vigorous frothing in the roller trough that the normal rate of flow of whey could not be maintained.

It was decided to transfer the experimental work to a small-scale roller drier. For comparison, data for a commercial plant and for this model drier are given below:

	Model	Factory
Steam pressure, lb./in.^{2}	40	50
Roller speed, rev./min.	15	16
Length of rollers, in.	24	80
Diameter of rollers, in.	12	28
Relative area of rollers	1	8½
Rate of whey, gallons/hr.	7	120
Preheating temperature of whey, ° F.	140	140

It was found in practice that, whereas on the commercial machine the dried whey is normally pushed off the knife by the bulk of following material, on the model machine, due to the thinner layer of drying material, the product usually formed a gradually broadening strip, separated from the rolls (and therefore not heated) but adhering at its upper end to the knife-edge [see Plate 1 (*b*)]. The colour, taste, texture and moisture contents of the two types of material did not, however, differ appreciably.

Using the small-scale drier, a number of different salts were added to batches of whey of varying acidities and the resulting liquid dried under the standard conditions noted above. The batches of whey employed varied from fresh whey of very low acidity (0·10 %) to stored whey of very high acidity (0·37 %). The results are summarized in Table 1. The following comments may be made.

The control sample dried satisfactorily. The addition of sodium bicarbonate confirmed the results already obtained on the large-scale plant. The product dried badly, was severely burnt, and frothed violently in the trough. In order to determine whether the faulty drying was due to the sodium ions *per se*, a similar run was made in which the sodium bicarbonate was replaced by sodium chloride. Apart from the absence of frothing, the effect was little better than with the bicarbonate.

It was considered possible that certain potassium salts might give a more satisfactory result than the sodium salts. Two such salts were used, i.e. potassium carbonate and di-potassium hydrogen phosphate. Both salts gave results at least as bad as with the sodium compounds.

Experiments were then made with calcium hydroxide in the form of a suspension of finely ground slaked lime. The results were in striking contrast to those obtained with the sodium and potassium salts, the drying properties, appearance and taste of the product made from the neutralized whey being

Table 1. *Cheese whey*

Neutralizing agent added to skimmed whey	Original acidity %	Acidity reduced to %	Moisture content of dried whey %	Drying properties	Taste of dried whey	Reconstituted whey (10 g. in 100 ml.)				
						*p*H	Acidity	Colour	Solubility 20° C.	Index‡ 50° C.
No addition	0·22	—	5·31	Good	Good	5·13	0·30	Good	99	100
$NaHCO_3$	0·20	0·12	6·34	Very bad. Browning at knife and violent frothing in trough	Burnt, rather lacking in taste	6·98	0·05	Muddy	102	102
NaCl (0·12 %)	0·23	0·23	5·21	Bad effect, but somewhat better than bicarbonate. No frothing	Very salty	5·57	0·25	Good	100	100
K_2CO_3	0·18	0·03	5·97	Very bad. Violent frothing, not drying at knife	Burnt, rather lacking in taste	7·05	0·04	Muddy	101	101
K_2HPO_4	0·37*	0·12	—	Very bad. Similar to bicarbonate, but no frothing	Better	6·56	0·15	Dark	—	—
,,	0·11†	0·06	—	,, ,,	,,	—	—	Very dark	—	—
$Ca(OH)_2$	0·10†	0·01	5·04	Good. Similar to untreated whey	Good	7·19	0·04	Dark	100	101
,,	0·17	0·05	—	,, ,,	,,	6·91	0·04	,,	—	—
,,	0·17	0·10	4·58	,, ,,	,,	6·37	0·11	Good	101	101
,,	0·18	0·05	—	,, ,,	,,	7·12	0·02	Dark	—	—
,,	0·20	0·06	—	,, ,,	,,	6·50	0·05	,,	—	—
,,	0·25	0·06	5·14	,, ,,	,,	6·70	0·03	,,	98	101
,,	0·30	0·06	3·66	,, ,,	,,	6·70	0·02	,,	99	100
,,	0·33*	0·05	—	Bad. Similar to bicarbonate, but no frothing	Sour and burnt	6·28	0·10	Very dark	—	—
$Ca(OH)_2$ (to over-neutralize)	0·20	Alkaline	—	Bad. Black product	Unpalatable	7·25	0·01	Black	—	—
$CaCl_2$ (0·12 %)	0·18	0·19	4·51	Rather worse than with lime, not as bad as bicarbonate or sodium chloride	Very salty	5·52	0·25	Good	96	98

* Whey kept 24 hr. at 20° C. (68° F.). † Drawn early. ‡ See Howat *et al.* (3).

as good as that made from freshly drawn whey of low acidity. This held for acidities which had been reduced from as high as 0·30 to 0·02 %. With only one sample (original acidity 0·33 %) was a poor product obtained, and this may have been due to some abnormal property of the whey itself, which had been stored at 20° C. for 24 hr. A check test with calcium chloride showed that calcium ions had a less deleterious effect than sodium ions.

Finally, one trial was made in which the whey was markedly over-neutralized with calcium hydroxide, i.e. was made actually alkaline. The resulting dried whey [which is illustrated in Plate 1 (*c*)] was an inedible, black product, in the drying of which the abnormalities experienced with partial neutralization by sodium bicarbonate were intensified (i.e. adherence to the knives, no formation of dry outer coat, and continuous "burning" on the rollers).

These experiments show, therefore, that (i) the use of sodium or potassium salts for neutralization produces with the roller process a very inferior dried whey, difficult to dry and consequently adversely affected in both appearance and taste; (ii) over-neutralization intensifies these defects; but (iii) correct neutralization with calcium hydroxide has no adverse effect either on the drying process or the quality of the dried product. It may, however, be noted that neutralization below an acidity of 0·05 % resulted in a slightly darker product.

2. The drying of acid casein whey

Two types of whey were used, one from casein precipitated with hydrochloric acid and the other from the acetic acid precipitation of casein. The wheys were prepared on a small scale, using the accepted procedure outlined by Sutermeister & Browne (1). The proximate composition of the two types of whey was almost identical, as shown by the following typical figures:

	Hydrochloric acid %	Acetic acid %
Total solids	7·1	6·9
Water	92·9	93·1
Lactose	5·5	5·2
Protein and salts	1·6	1·7
Fat	Nil	Nil
Acidity (as lactic acid)	0·47–0·50	0·40–0·42

The reactions of these two types of whey to roller drying were, however, very different. Each type will therefore be dealt with separately.

(i) *Hydrochloric acid casein whey*

Preliminary trials showed that, as with strongly acid cheese whey, the unneutralized whey of 0·47–0·50 % acidity could not be successfully dried by the roller process. Following the work already described, sodium or potassium salts were avoided and attempts were made to neutralize the liquid prior to drying with calcium hydroxide.

It was found, however, that complete neutralization with lime resulted in

the flocculation of a bulky precipitate, the composition of which when dried was as follows:

	%		%
Water	3·9	Protein	60·5
Ash	17·4	Lactose	15·7

It may be noted that Richter(2) obtained a similar precipitate when neutralizing sulphuric acid casein whey. It has been suggested that this dried by-product might be used as a supplementary form of feeding stuff for poultry and other stock.

The effect of the presence of this precipitate during roller drying was very unfavourable. Table 2 gives the results of a series of trials in which varying quantities of lime were added to the whey. It will be seen that progressive neutralization to an acidity of 0·18 % gave increasingly satisfactory drying properties. Further neutralization was, however, accompanied by precipitate formation with a marked deterioration in drying properties. The flocculent precipitate passed from the roller trough on to the roller surfaces, covering them with irregular flocks or patches of white coagulated material. At the same time the whey film collected and revolved at the knife-edge without drying, becoming progressively darker in colour [see Plate 2 (*a*)].

It will be noted from Table 2 that the reconstituted product invariably had an acid and bitter taste. In general it can be described as definitely unpalatable, and it is questionable whether it would even be relished by live stock unless well masked in a food mixture.

(ii) *Acetic acid casein whey*

It has already been noted that the proximate composition of whey from acetic acid casein is practically identical with that from hydrochloric acid casein. The drying properties were, however, found to be entirely different, as will be seen from Table 3. Although the initial acidity of the whey was 0·40–0·42 %[1] no difficulty was experienced in drying without the addition of a neutralizing agent [Plate 2 (*b*)], and the final acidity (determined in the reconstituted product) was found to have fallen to 0·06–0·08 %. The acetic acid had apparently been vaporized by contact with the heated rollers during the period of heating in the roller trough, so that the liquid behaved in a manner similar to low acidity cheese whey. This explanation receives interesting confirmation in the fact that, when calcium hydroxide was added prior to drying in order to reduce the initial acidity of the whey, the dried product was found to be markedly alkaline, i.e. vaporization of free acetic acid had taken place and the presence of excess of the undissolved suspension of lime had resulted in the over-neutralization of the powder.

It would appear possible, from the results shown in Table 3, to arrange for the addition of lime in quantities just sufficient to neutralize the residual (non-volatile) acidity. It was in fact found that the addition of 0·4 % of lime

[1] With acetic acid this acidity was found to be sufficient for precipitating all the casein from skim milk at 110° F. (44° C.). With hydrochloric acid the acidity required was 0·45–0·50 %. Slow preheating of the acid whey above 80° C. causes precipitation of the protein.

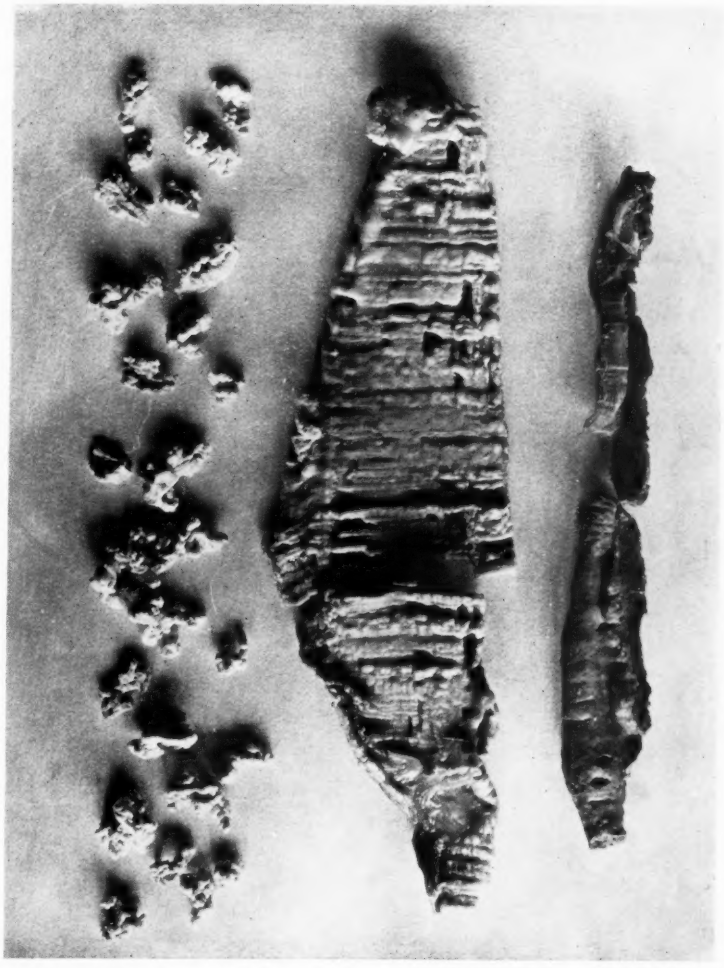
Table 2. *Hydrochloric acid whey*

Original acidity %	Reduced with lime to %	Moisture content of dried whey %	Drying properties	Reconstituted whey (10 g. in 100 ml. water) Acidity %	*p*H	Taste	Colour	Solubility 20° C.	Index 50° C.
0·48	—	4·69	Bad, burning at roller	0·50	4·12	Very acid	Brown	100	101
0·47	0·37	—	Fairly good	0·36	5·12	Acid and bitter	Yellowish-white	—	—
0·48	0·32	4·09	Good	0·31	5·27	,, ,,	,, ,,	95	95
0·49	0·18	3·61	Very good	0·17	5·89	Slightly acid and bitter	,, ,,	100	101
0·49	0·16	—	Fairly good, slight tendency to revolve at knife	—	—	,, ,,	,, ,,	—	—
0·44	0·14	—	Moderate, tendency to revolve at knife. Slight precipitate	—	6·00	,, ,,	,, ,,	—	—
0·49	0·10	5·17	Bad precipitate, revolving at knife-edge and burning	—	6·31	,, ,,	Increasingly burnt appearance	91	92
0·49	0·06	—	Not attempted. Large precipitate	—	—	—	—	—	—

Table 3. *Acetic acid whey*

Original acidity %	Reduced with lime to %	Moisture content of dried whey %	Drying properties	Reconstituted whey (10 g. in 100 ml. water) Acidity %	*p*H	Taste	Colour	Solubility 20° C.	Index 50° C.
0·42	—	4·12	Excellent	0·06	6·73	Very good	Greyish-brown at first; on standing, yellow	99	99
0·40	—	—	,,	0·08	6·63			—	—
0·40*	—	—	,,	0·08	6·61			—	—
0·40	0·29	6·18	,,	0·02	6·98	Good but slightly stale		96	96
0·42	0·25	5·54	,,	Alkaline	7·70			97	98
0·42	0·15	—	,,	,,	7·70			—	—

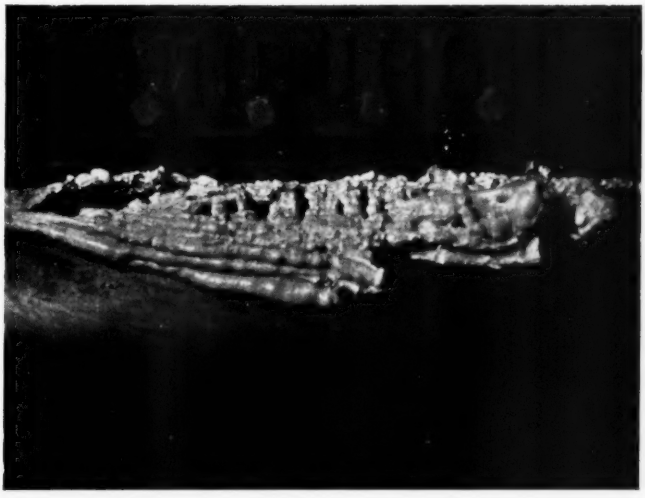
* Kept 24 hr. before drying.



(*a*) (*b*) (*c*)

(*a*) Dried whey from a commercial roller drying plant. Note "popcorn" appearance. (*b*) Ditto from the model plant. Note the failure to break up into fragments, but the satisfactory light appearance of the dried sheet. (*c*) Ditto, but made from over-neutralized whey. The molten mass has continuously revolved at the knife-edges, giving a solid cylindrical mass of burnt material.

(*a*) A partially neutralized hydrochloric acid whey not drying satisfactorily. Flocks on the roller due to precipitate; undried mass revolving and burning at knife.



(*b*) An unneutralized acetic acid whey drying excellently. Note clean rollers, dried whey about to fall from knife.

to whey of 0·40 % acidity produced an exactly neutral dried whey. A further point of interest is that the samples of acetic acid casein whey which had been kept for 24 hr. before drying showed as good drying properties as the fresh whey. Moreover, the lactose content had not fallen, as is the case with hydrochloric acid casein whey.

The reconstituted whey from all the acetic acid casein whey powders (particularly from the unneutralized whey) was quite palatable and the taste very similar to that of ordinary cheese whey. The usual saltiness of whey was, however, naturally lacking.

Thus it will be seen that it is very much easier to dry the whey obtained by precipitating casein with acetic acid than that resulting from the hydrochloric acid precipitation. With the former, unneutralized whey can be fed directly to the rollers to give an easily dried product of good appearance, very low acidity and palatable taste. With hydrochloric acid whey, lime must be added to reduce the acidity to a critical limit (which is easily over-stepped) below which a precipitate is formed and the drying properties ruined, while the taste of the partially neutralized dried whey is distinctly acid and bitter.

SUMMARY

1. Experiments have been carried out in order to determine the best method of neutralizing cheese whey prior to drying by the roller process. The use of sodium or potassium compounds leads to difficulties in drying and the production of an unpalatable product of poor quality. Neutralization with calcium hydroxide gives a satisfactory product. Over-neutralization must be avoided.

2. Complete neutralization of hydrochloric acid casein whey results in the formation of a flocculent precipitate and deterioration in the drying properties; reduction of the acidity from 0·48 to 0·18 % by the addition of calcium hydroxide allows successful drying and yields a reasonably satisfactory product. The latter is, however, definitely unpalatable.

3. Acetic acid casein whey can be dried without any previous neutralization to yield an excellent and palatable product.

ACKNOWLEDGEMENTS

Thanks are due to Messrs Bertram's of Edinburgh for the loan of the small-scale roller drier and to Mr James Kirkwood of the West of Scotland Agricultural College for providing the cheese whey and for assistance in preparing the acid casein whey.

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(*MS. received for publication* 12 *December* 1940)

BIENNIAL REVIEWS OF THE PROGRESS OF DAIRY SCIENCE

SECTION A. PHYSIOLOGY OF DAIRY CATTLE

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I. REPRODUCTION AND LACTATION

An estimate made some years ago showed(1) that in Britain about one-quarter of the wastage in dairy herds could be attributed to sterility in one form or another. More recent figures which are available for Finland(2) suggest that in that country over 36% of the cattle culled from dairy herds, whether of the Ayrshire or local breeds, are rejected for this same reason. Since much of the sterility in the bovine is functional rather than pathological, it is natural that the physiological aspects of a subject, so all-important to the dairy farmer, should continue to be studied in many research schools. For a clear and concise account of recent advances in this direction, reference should be made to a paper read by Hammond(3) at the Annual Congress of the National Veterinary Medical Association. The paper was very fully discussed by a number of prominent authorities and details of the discussion are published. Various factors, such as inadequate exercise, anatomical and functional hypertrophy of the mammary gland, hormone control and nutrition, were debated.

For an excellent guide to present-day knowledge on the subject of nutrition as it affects fertility and reproduction, the reader should refer to an article by Crowther(4) in which the requirements of energy, protein, vitamins and minerals are lucidly discussed. The subject-matter is presented in a way which will be of much value to those interested in the practical aspects of animal husbandry. General articles have also appeared on the same subject by Campbell(5, 6), and by McIntosh(7, 8). The latter deals particularly with diets deficient in phosphorus and vitamin A.

The results of original work carried out during the past few years on the feeding of cattle in connexion with reproduction have been published recently by a number of authors. Smirnov-Ugrjumov & Laptev(9, 10) report that liberal supplies of green foods and protein, particularly protein of animal origin, such as that contained in blood meal or skimmed milk, are most efficacious in increasing sexual activity and spermatogenesis in bulls. One reason for the value of green foods in this direction may be their content of vitamin C. The essential nature of this vitamin for fertility in the bull has been investigated by Phillips & Lardy(11), who claim that lost fertility could frequently be restored by subcutaneous injection of ascorbic acid. They found that potent bull semen normally contains 1·5–3·5 mg. of this vitamin per 100 ml., and that values below 1 mg. and greater than 4 mg. were associated with very poor fertility and most unreliable breeding records. Owing to the importance of vitamin E for reproduction in some species it is natural that this factor should also be studied in connexion with bovine fertility. The view is generally held, however, that although vitamin E is probably an essential food factor for normal reproduction in farm animals, it is never likely to be of importance in actual practice, since it is so prevalent in much of the food normally fed on the farm. A paper by Underbjerg *et al.*(12) confirms this view in regard to the dairy goat.

A general discussion on sterility as it is affected by such factors as climate, nutrition and endocrinological conditions has been published by Löwe(13). The

treatise extends to some 200 pages and contains thirty-nine diagrams. It cannot therefore be treated adequately in the present brief review. By statistical analysis, the author concludes that there are differences in fertility between different cow families which cannot be explained except by assuming the existence of important hereditary factors.

A brief summary of hormone treatments for combating sterility has been published by Asdell(14). Should sterility be due to infantile ovaries, a favourable response frequently occurs on administering anterior pituitary extracts. Persistent corpora lutea, however, do not appear to respond to hormone therapy. Where sterility is due to non-retention of the ovum by the uterus, the administration of an oestrogen to increase the vascularity of the uterine mucosa might be expected to yield valuable results. It has been found by Asdell, however, that, on administering Progynon B (a commercially prepared oestrogen) to a group of cows, the number of conceptions was not significantly greater in this group than in the group to which no hormone was given. Further work will be required before any definite conclusions can be drawn.

Goerttler(15) discusses sterility as it exists in the cattle of Thuringia, while Tutt(16) warns owners of Guernsey cattle that their breed may ultimately become extinct unless inbreeding is checked.

The effects of age and season of the year on reproduction have been studied by several workers. A report by Morgan & Davis(17) contains an analysis of records kept between 1896 and 1934 for over 2000 Nebraska cattle. They found that the ratio of services to conceptions was least for bulls under 2 years old, and that it varied little between the ages of 2 and 8 years. The average number of services required for each conception was 2·21. This value varied with the season. It was minimal in December at 1·94 and greatest in September at 2·86. Dawson(18) found a mean ratio of 2·49, but points out that his figure may be slightly high because in his herd sterile cows were kept longer than usual. In Dawson's experiments the ratio varied slightly but not significantly with the season. With regard to age, he found that individual bulls varied so much from one 6 months' period to another that averages for various animals could not readily be interpretated, nor could they reasonably be applied to separate individuals.

As a criterion of the fertility of bulls Walton & Edwards(19) have suggested the measurement of the respiration rate of the sperm. They found, under the particular breeding conditions in their herds, that the higher the respiration rate, the fewer were the number of services required for conception. When the ratio of services to conceptions was under 2, the respiration rate was represented by the value 0·29; when it was between 3 and 7, the corresponding value was 0·13.

The morphological and physiological properties of the sperm from Brown Swiss bulls have been studied by Sciuchetti(20). He confirms that the morphology and variation of the sperm heads are the best indication of fertilizing properties, but states that the number and motility of the spermatozoa and the volume of the ejaculate may also have to be considered. He found that with up to 25% of abnormal sperms fertility was low, with 26–35% it was reduced, and with over 36% it was very poor. Morphological variations in bull sperms have also been discussed by Swanson & Herman(21). The mode of migration of spermatozoa has been investigated by Tassinari(22), who examined portions of the genital tract of cows slaughtered a few hours after insemination. He concludes that the progression of the spermatozoa is chiefly due to their own motility, aided to some extent by the motion of the epithelial cilia.

It is often believed that in certain years the number of bull calves born greatly exceeds the number of heifers, while in other years the reverse may be true. Stolberg-

Wernigerode(23) has disproved this belief by an analysis of records kept for three herds between 1930 and 1939 when 1704 calves were born. In the three herds the proportion of births resulting in bull calves was 54·5, 50·5 and 52·7% per year. Morgan & Davis(17) state that the average proportion of males born in their herds during a period of many years was 53·75%. The sex ratio of calves in the foetal state has been discussed by Chapman *et al.*(24).

The diagnosis of pregnancy in the cow has been reviewed by Cuboni(25). It is generally held that methods involving hormone estimation or detection, such as gonadotropic tests on blood and urine and oestrogen tests on blood, are not possible with the cow, the only test of any promise being that for oestrogen in the urine. Cuboni confirms this view and shows that the fluorescence test which he used for mares fails when applied to the urine of cows. Dickenschield(26) obtained a correct diagnosis in only 47% of the cases tested. Cuboni has improved a method previously put forward by Josef(27) for estimating oestrogens in urine and claims 98% accuracy for the diagnosis of pregnancy in cows after 5 months. It is unfortunate that a reliable diagnosis cannot be made at an earlier stage when the result would be of so much greater importance to the breeder. A method for estimating oestrogens in urine has also been discussed by Tang *et al.*(28).

With regard to the length of the gestation period in cattle, Marval(29) suggests from 4 years' records that the generally accepted time of 281 days is too short. The average found by him for 150 calvings was 284·5 days. The average time for Caracu cattle has been discussed by Jordao & Veiga(30), who have kept records of 980 pregnancies during 28 years. The average duration was 286·9 days, 54% of the pregnancies falling into the 280–290 days' group and 29% into the 290–299 days' group. The age of the cow appeared to have no significant effect on these values.

Multiple births in cattle have been fully dealt with by Löwe(31), who has statistically analysed the occurrence of such births in certain large cow families in relation to non-hereditary factors such as age, season and the interval between calving. Should it be desirable to avoid multiple births owing to the higher death-rate of the calves, the author recommends that the first mating should take place before the animal is 24 months old, since later mating appears to favour the occurrence of multiple births. A large number of relevant factors are considered in detail. Perhaps it may be of interest to mention here an extraordinary instance of fecundity reported by Bichat(32) in which a ten-year-old cow gave birth to five calves in one year, i.e. quadruplets followed by a single calf 11 months later. One of the quadruplets died at birth.

The effect of the time of calving or of conception and the interval between calvings on milk and milk-fat production have been studied by numerous workers. Edwards(33) claims that in Georgia and Maine the future butterfat-producing capacity of calves tends to be greater when conception occurs in the cool seasons of the year. He believes that climatic factors (possibly affecting the endocrine system) tend to be responsible for this rather than differences in pastures and feeds. Baron-Hay & Slater(34) have found that in Western Australia the maximum yield of butterfat averaged 260 lb. for cows calving in April as compared with 200 lb. for those calving in September. On the other hand Hewitt(35), in Victoria, obtained no significant difference in production as a result of changes in the time of calving and in the interval between calvings, but he recommends a dry period of at least 3 weeks for Friesians and 6 weeks for Red Polls. Ungelenk(36) in Germany recommends a minimum dry period of 8 weeks. For cows in the Philippines, Rigor(37) has obtained higher yields from those calving during the dry season than during other times of the year, and records that a short dry season favours greater production.

Hormones

Two excellent descriptions of the part played by hormones in the development of the mammary gland and in the initiation and maintenance of lactation have appeared recently. One is a general review by Folley (38), and the other a chapter on mammary glands published by Turner in a new edition of *Sex and Internal Secretions* (39). The inclusion of a section on hormones in the present review might therefore at first sight seem superfluous. The object of the present writer, however, is not to present an account of the subject in its entirety but to discuss only those advances which have been made in the subject during the past 2 years.

So far, no theory has been put forward which will account for all the recorded observations published in recent years, and many important points still await elucidation. The following conception, formulated principally by Turner and his colleagues at Missouri, forms, however, a suitable foundation for the discussion which follows. It is believed by these workers that in many species oestrogens secreted by the ovaries stimulate the anterior lobe of the pituitary to secrete a factor which induces duct growth in the mammary glands and which has been named Mammogen I. During pregnancy progesterone from the corpus luteum also acts, together with the oestrogens, on the anterior pituitary, causing it to secrete Mammogen II, a hormone which is believed to bring about development in the milk glands of the lobule-alveolar tissue. The mammary gland is thus prepared during pregnancy for the subsequent lactation. At parturition, the anterior pituitary provides certain lactogenic hormones which are responsible for the initiation and maintenance of the milk secretory processes. Recent work bearing on these theories will now be discussed.

The pituitary hormones. The suggestion that the lactogenic hormone of the pituitary cannot be regarded as a single substance has been made in recent years by Folley & Young (40), who have studied the effect of various anterior pituitary extracts on established lactation in the cow. They have shown that it is possible to prepare extracts from the pituitary which possess the power of stimulating pigeon-crop growth without exhibiting lactogenic properties. They suggest that the term "prolactin" should be reserved for extracts having this type of hormonal activity, irrespective of whether such extracts are actually lactogenic or not. These authors studied the effect of a single injection of various different extracts. Their results may be summarized as follows: (1) A crude saline extract of fresh pituitaries, (2) a prolactin fraction prepared at 0° from fresh glands, and (3) a thyrotropic fraction also prepared at 0° from fresh glands but containing no prolactin activity, all caused marked increases in yield of 5 or 6 days' duration. When, however, prolactin and thyrotropic extracts were made at room temperature from commercially acetone-dried glands and single doses of these preparations equivalent in prolactin and thyrotropic activities to (2) and (3) were injected, no corresponding increase in milk yield resulted. Since one of the main differences between the first three extracts and the last two was that the former were rich in the glycotropic ("anti-insulin") factor known to be present n the pituitary, the ability of these various extracts to increase milk yield was correlated with the presence of a glycotropic factor and not with prolactin activity as measured by the pigeon-crop gland or with thyrotropic activity. The authors point out that the glycotropic hormone may well be a limiting factor in lactation since it opposes any tendency to a diminished blood sugar level, particularly since there is evidence to suggest that milk yield and lactose content may depend to some extent on the level of glucose in the blood (Graham *et al.* (41)). Folley & Young also injected large doses of their prolactin preparation from dried glands into one cow daily for 15 days. They found that there was a marked increase in milk yield of higher lactose content and that this enhanced yield persisted for a month or more

after the last injection. They suggest that this treatment allayed the natural involution of the gland or even rejuvenated some of the lobule-alveolar tissue, thus permitting the increased yield of milk. At the present time when the yield of home-produced milk and milk products is of vital importance to the nation, it is of interest to observe that by the injection of their prolactin preparation, Folley & Young obtained extra milk which could have been sold for twelve shillings, while the extract they used cost them eighteen pence. Even taking into consideration the extra feeding stuffs for the animals and the cost of labour to make the extract, some such procedure might well become of practical importance during periods of food shortage.

In experiments described in a later paper the same authors(42) have injected prolactin and thyrotropic preparations rich in the glycotropic factor into groups of cows. The prolactin group showed a marked rise in milk yield of enhanced fat content, so that an average increase of 48% in fat secretion per day was observed during the period of five successive injections. The increase in yield was not so great with the thyrotropic preparation and the fat content was unaffected. No significant alterations with either extracts were found in the content of lactose, chlorides, non-fatty solids and phosphatase. The composition of the milk was therefore normal during the period of enhanced yield, a fact which is most important if hormone therapy is ever to be of practical value in milk secretion problems. In this connexion it has also been shown by Jacobsen(43) for rabbits that prolactin treatment has no adverse effects on milk composition.

The activity of pituitary lactogenic preparations with respect to both their ability to stimulate growth of the pigeon-crop gland and also to initiate lactation in the rabbit has been studied by Bergman *et al.*(44). They found that a highly active preparation, almost free from other known pituitary hormones, showed the same relationship between pigeon and rabbit units as was shown by a crude anterior lobe extract. These authors therefore believe that the crop-stimulating factor and the principal lactogenic factor are identical and suggest that the term "lactogenic" should be reserved for a substance which actually *initiates* lactation. A similar conclusion has been arrived at by Evans(45). Folley & Young(46) have published the results of some further experiments on the continued treatment of lactating cows with anterior pituitary extracts. Their earlier work(40) had shown that when cows received regular injections of prolactin for 2 or 3 weeks the initial increase in milk production was followed by a decline even during continued treatment. The authors have now endeavoured to find whether this decline could be ascribed to the development of some "anti-hormone" inhibiting the action of prolactin. No evidence of this was obtained, however, nor did it seem likely from the composition of the milk that the decline in lactation was due to a diminished supply of the required milk precursors. In this work it was again found, contrary to the results of Bergman *et al.*(44), that the ability to stimulate milk production was not correlated with prolactin activity. Thus a crude pituitary extract was three times more potent in stimulating milk production than an extract possessing seven times as much power to stimulate crop proliferation. Folley & Young suggest that, since Bergman and his colleagues wish the term "lactogenic" applied only to substances which *initiate* lactation, the term "galactopoietic" should be applied to an anterior pituitary extract which actually stimulates *increased* production in established lactation. Thus, in these latter experiments of Folley & Young, prolactin preparations low in glycotropic activity were also low in galactopoietic potency. Contrary to the findings of the earlier work(40), however, it is now emphasized from the more recent results that galactopoietic activity does not appear to be correlated solely with glycotropic power, but that both glycotropic and prolactin activity appear to be essential.

The separation of hormones with prolactin activity from the carbohydrate metabolism factor of the pituitary has been described by Bergman & Turner(47), who extracted the glands with alcohol and precipitated the extract with Na_2SO_4 and H_2SO_4 at *p*H 3·5. The precipitate on further purification contained 6666 pigeon units of prolactin and only 10 guinea-pig units of the carbohydrate metabolism hormone per g. A further precipitate was obtained by the addition of flavianic acid at *p*H 4·5 to the liquid portion resulting from the first precipitation. On further purification this latter substance was found to contain 500 guinea-pig units of the carbohydrate hormone per g. and only 10 pigeon units of prolactin. The activity of both extracts was about 100% more than that of the initial material.

Further evidence that prolactin is not the only pituitary factor involved in the maintenance of milk secretion has been published recently by Reece(48), who found that prolactin preparations were not effective in initiating lactation in pseudo-pregnant rats, but that their activity could be increased in this direction by the simultaneous injection of an extract from the adrenal cortex. The prolactin extracts were, therefore, deficient in some other lactogenic hormone which is capable of becoming a limiting factor, at least with pseudo-pregnant rats. Further work by Reece *et al.*(49), in which pituitary glands of pregnant rats were assayed at various intervals after parturition, showed that the prolactin activity decreased from 10·5 bird units per gland at 2 days *post partum* to 8·3 units 21 days later. They conclude that, while prolactin appears to play no part in determining the height of the lactation curve, it may to some extent control the rate at which the milk yield declines. Reece(50) has also estimated the prolactin activity of female guinea-pig pituitaries at various stages of the sexual cycle. He found that the concentration was greatest during lactation and that in descending order of magnitude the various stages could be arranged in the following sequence: late pregnancy, oestrus, early pregnancy and dioestrus. A somewhat similar experiment has been made by Holst & Turner(51) with lactating and pregnant rabbits and guinea-pigs. With rabbits a slight rise in prolactin took place just prior to parturition and reached a maximum 5–10 days *post partum* when the pituitaries were twice as heavy as those of non-lactating animals. There was then a gradual decline.

In order to find the most suitable source of material for the extraction of various anterior pituitary hormones, the content of these hormones in the anterior pituitaries of different species has been determined by Chance *et al.*(52). The relative figures for prolactin in different species were for sheep 100, ox 94, man 37, pig 5 and horse 4. The extraction of prolactin from the liver of the ox and pig has been studied by Rabald & Voss(53). A preparation was obtained which contained only 5% of the activity per unit weight of a similar preparation made from the pituitary, but from the liver the total yield per animal was 100 times greater than from the hypophysis.

The actual site of formation of the pigeon-crop stimulating hormone has been studied by Azimov & Altman(54), who separated pituitary tissue into eosinophilic and basophilic zones and endeavoured to extract prolactin from both. The eosinophilic tissue gave rise to a preparation which was not only more active in stimulating milk secretion in cows than that from the basophilic but was also more potent than a similar extract from the whole anterior lobe. It is therefore thought that the eosinophilic zone may be the site of formation of prolactin.

A few years ago White *et al.*(55) succeeded in isolating a crystalline protein which possessed high crop-growth stimulating activity but which appeared to be free from growth-promoting, thyrotropic, gonadotropic and other pituitary hormones. The chemical properties of this prolactin preparation have now been further studied by White & Bonsnes(56). According to White & Lavin(57) its ultra-violet spectrum has

a band with maximum absorption at about 2800° A. Another preparation has been examined by Li *et al.*(58, 59). The phosphorus-free substance contained some 0·53% amino-nitrogen, and it was shown that when these amino-groups were acetylated by treatment with ketene or removed by the action of nitrous acid the prolactin activity of the substance was destroyed. The importance of the amino-groups was confirmed for another preparation by Bottomley & Folley(60), who formed a phenyl-ureido derivative with phenyl isocyanate. The essential nature of the amino-groups for prolactin activity is particularly noteworthy in view of the fact that insulin, which is a somewhat similar protein, has been found by Stern & White(61) to retain its activity even after acetylation with ketene. The "crystalline" preparation of prolactin isolated by Lyons and his colleagues has been tested for homogeneity by Li *et al.*(62, 63) by means of electrophoretic technique, while White's sample has been studied in the same way by Shipley *et al.*(64). This method, in which 0·5% protein solutions were tested and in which 0·02% solutions of protein can be detected, showed that in both preparations there could not have been more than exceedingly small amounts of contaminating substances. The experiments suggested, however, that the two crystalline proteins were not identical. Storage of solutions of White's preparations appeared to result in denaturization and the formation of a second protein. Immunological investigations on pituitary lactogenic preparations have been carried out by Bischoff & Lyons(65), Young(66) and Strangeways(67).

Reece *et al.*(68) have published an account of experiments on the effects of diets deficient in vitamins A, B, C, D and E on the prolactin content of rat pituitaries. Only with vitamin D deficiency did there appear to be any change which could definitely be interpreted as a reduction.

The effect of prolactin on milk secretion in women has been investigated by Stewart & Pratt(69), but it is impossible to arrive at definite conclusions from the results. With human subjects the difficulty of securing adequate controls and of knowing whether deficient lactation would have become normal without hormone therapy are very great.

With regard to the assay of prolactin, the results of different methods used in various laboratories have been examined by Emmers(70) and a memorandum on the international standard for prolactin has been published. Since the appearance of this memorandum, a paper which will be invaluable to those who have occasion to estimate prolactin has been published by Folley *et al.*(71). By the statistical analysis of the results of many prolactin assays which they have carried out by the pigeon-crop weight method, the authors have been able to make a number of important recommendations which must be followed if a reasonable degree of accuracy is to be obtained. A dose-response curve has been constructed and it has been shown that for doses of 3–18 international units a very close approximation to a rectilinear relationship exists between crop weight and the logarithm of the dose, the relationship being even closer when the crop weight is expressed as a percentage of the body weight. The authors recommend that such a curve should be obtained for each laboratory carrying out assays of prolactin and that a simultaneous comparison between the unknown and the standard preparations should always be made. It is also important that there should be 15–20 pigeons each weighing 260–360 g. in each group, that the mean weight of each group should be as nearly equal as possible, and that the birds should be kept at one temperature, preferably 15° C. A further precaution is that the injections should be subcutaneous and not intramuscular or intradermal, since the latter both produce less response. Further work on the assay of prolactin by Bates & Riddle(72) shows that the volume of water used for injecting the hormone has a most marked effect on the result. Thus when a given quantity of preparation was dissolved in 0·5 ml. and injected, its apparent effectiveness was four

times as great as when it was dissolved in only 0·05 ml. A constant volume of fluid must therefore be used for the assay of this hormone.

The mammogens of the anterior pituitary. Much evidence has accumulated during the past 2 years for and against the presence in the pituitary of a mammary duct growth-stimulating hormone to which the name "Mammogen I" has been given by Turner and his co-workers. The earlier evidence for the presence of this hormone (73) was cited in the previous review of this series (74), but it has recently been disputed again by Nelson (75, 76), who states that on implanting pituitaries from oestrogen-treated rats of either sex on hypophysectomized immature female rats or male mice, the mammary development did not exceed nor even equal that which was obtained by implanting normal pituitaries. Further evidence against the existence of this hormone has been published by Lyons & Sako (77), who have rubbed into the skin of the rudimentary mammae of young male rabbits suitable doses of oestrone in oil. Certain glands rubbed with the oil only showed negligible growth, whereas those which were treated with oestrone developed considerably. It would therefore appear that the action of oestrone was direct and local and that it did not involve the liberation from the pituitary of a specific hormone which would circulate in the blood and thus be available for the stimulation of all the glands. Work on certain synthetic sex hormones by Noble (78) also suggests that at least a portion of the action of these substances on the mammary gland may be direct and not through the pituitary. Thus when ovariectomized rats were given testosterone propionate, growth of the nipples, ducts and lobule alveolar tissue took place. When, however, the rats were both ovariectomized and hypophysectomized only nipple growth without further development could be induced. Noble concludes that in the absence of both the hypophysis and ovaries testosterone propionate causes growth of the duct system only and that for further development the pituitary is essential.

The fact that the body-growth hormone of the pituitary may be a limiting factor in the development of the mammae under oestrogenic treatment has been suggested by Nathanson *et al.* (79), who found that when oestradiol benzoate was given alone to a group of hypophysectomized rats, almost complete atrophy of the mammary glands resulted. When, however, the growth hormone was given in addition, out of twenty-four animals eight increased in weight and showed marked development of the mammae, six showed no increase in weight and only moderate mammary development, while the remaining ten which lost weight showed either atrophy or very poor development. The degree of mammary development in the eight hypophysectomized animals just mentioned was equal to that of a normal control group which received no treatment, but it was inferior to that of a group of intact animals receiving oestradiol benzoate. The authors conclude therefore that the growth factor, though essential for full normal development, is not the only hormone involved, and that apart from this the action of oestrogens in developing the mammae requires the presence of the pituitary.

Because some of the views just cited were so much in conflict with those of Turner and his colleagues, Reece & Leonard (80) have implanted pituitaries from normal and oestrogen-treated rats of both sexes into a group of hypophysectomized and ovariectomized immature rats, one such group being kept untreated as a control. At the end of the treatment, the mammae of the controls had involuted, whereas those of the rats which had received implantations from both normal and oestrogen treated animals showed considerable development. This work, therefore, provides evidence in favour of the conclusion that there is a factor in the pituitaries of both sexes capable of stimulating growth in the milk glands of immature hypophysectomized castrated rats. It should be noted, however, that implantations from normal rats were effective as well as those from the oestrogen-treated animals.

Further evidence in favour of this theory has now been published by the Missouri Research Station. Lewis & Turner(81), for example, have extracted fresh pituitary tissue from pregnant cattle with alcohol and have precipitated the prolactin and carbohydrate metabolism hormones in 86% alcohol. On evaporation of the alcohol *in vacuo* an oily residue containing high duct-growth activity was obtained. Since this hormone is soluble in fat solvents, it is evidently entirely different in chemical constitution from prolactin and other anterior pituitary hormones. A method for its biological estimation has now been proposed by Lewis *et al.*(82), who suggest that the unit of assay should be the amount of active material required to produce definite signs of development in one or more glands of $50 \pm 10\%$ of ten or more male albino mice weighing 10–25 g. Injections should be made once daily for 6 days and the animal killed on the seventh day. A complete account of their work on Mammogen I has been given by Lewis & Turner(83) in a special bulletin. This publication, which s well illustrated and excellently produced, is worthy of a far more detailed account than can be given here. It contains a description of the assay of the hormone in 545 pituitary glands obtained from lactating cows, heifers, steers and bulls at different ages and stages. Throughout pregnancy the pituitaries of dairy cows were found to be richer in mammogen than those of pregnant beef cows, which might be taken as suggesting in part a reason for the larger and more fully developed udders of dairy cows as compared with those of beef cows. Several findings of this nature are recorded in the bulletin and a valuable bibliography of the literature supplying evidence both for and against the existence of the mammogenic hormone is included. In connexion with the assay of this factor, Lewis(84) has pointed out that the technique may readily be used for the assay of both natural and synthetic oestrogens which stimulate duct growth and that the results obtained in this way form a more reliable measure of the mammary growth potency of the oestrogens than comparisons made on the basis of genital response.

Mixner(85) has made a preliminary report on the existence of Mammogen II, the hormone which is believed by some to be necessary in many species for the proliferation of the lobule-alveolar tissue. Apparently this hormone differs markedly from Mammogen I in being protein-like in nature, with the result that the separation of the two has been fairly readily attained. The assay of this factor has also been studied, and the unit is tentatively defined as the amount of material required to be injected daily for 6 days to secure definite lobule-alveolar development in $50 \pm 10\%$ of ten or more castrated, nulliparous female mice weighing between 15 and 20 g. It has been shown that in one instance 125 mg. of pregnant-cattle pituitary contained 1 mouse unit of activity, whereas as much as 600 mg. of the same tissue from non-pregnant cattle failed to show any activity whatever. This result supports the theory that during pregnancy mammogenic hormones secreted by the pituitary are essential for mammary development.

Results bearing on this subject have recently been published by Desclin(86). He found that when male guinea-pigs, in which the mammae had been developed by oestrogens, were hypophysectomized and oestrogen treatment continued for a further 12 days, the mammary glands had completely atrophied except for the ducts and areoles, thus suggesting that the pituitary is essential for the glands to remain actively developed under the action of oestrogens. He also removed the pituitary from female guinea-pigs after the fortieth day of pregnancy and found that 9 to 12 days later the mammae showed no signs of atrophy. He believes that when the maternal pituitary is removed either the foetal pituitary or the placenta can act in its place.

The thyrotropic hormone of the pituitary. The amount of thyrotropic hormone in the pituitaries of rats at various stages of development has been estimated by Turner & Cupps(87), using the method of assay which depends on the injection of the material

to be tested into day-old chicks. During the first half of pregnancy there was little increase in the content of thyrotropic hormone, but towards the end of the gestation period a substantial rise was observed. This was followed by a further increase of some 40% after the initiation of milk secretion, but there was no significant trend in the concentration of the hormone during the remainder of the lactation. The authors consider that their results, taken together with those of Reece & Turner (88) on cattle, suggest that an increased supply of the thyrotropic hormone is one of the factors concerned in the rising portion of the normal lactation curve. The influence of this hormone on the yield and composition of goats' milk is discussed in a thesis by Teggenthien (89). A description of the detailed results of these experiments is not available to the reviewer, but from an abstract of the thesis it appears that the milk yield diminished by about 10% during the hormone treatment and that the fat content rose to 4·5% as compared with a value of 2·5% observed between doses. There was therefore an increase in total milk fat secretion as a result of the hormone treatment.

The assay and purification of the thyrotropic hormone have been discussed recently by Traenkel-Courat *et al.* (90) and also by Bergman & Turner (91). The latter have compared the guinea-pig and chick methods of assay.

The sex hormones. So many papers dealing with the hormonal activities of both the natural and synthetic oestrogens have been published during the last few years that it will only be possible to deal briefly in the present review with those which appear to be of more or less direct importance in studies on dairy cattle. There has never been entire agreement on all the points at issue between the various workers in this field as to the role of the sex hormones in lactation, but it is undisputed that they are instrumental in causing the development of the mammary gland before lactation begins. Their effect once lactation has been established is less clear. With certain animals, large doses tend to inhibit lactation; with others the administration of smaller amounts can under suitable conditions improve the quality of the milk without decreasing the yield.

Recently, de Fremery (92) has reported that, whereas regular milking brought about no mammary growth or milk secretion in virgin goats, the administration of oestradiol benzoate either by injection or inunction caused rapid udder development. Regular milking, however, produced no actual secretion unless a few prolactin injections were also given, after which the induced lactation lasted for some months. As would be expected, prolactin itself did not produce secretion without previous oestrogen treatment. When the oestrogen was given after the lactation had been established, a decrease in yield took place and the composition of the milk approached that of colostrum, effects which were reversed on cessation of the oestrogen administration. A few years ago Folley (93) obtained a marked enrichment of cows' milk by oestrogen treatment during established lactation. He and his colleagues (94) have now studied the value of the much more readily available synthetic oestrogen, diethyl stilboestrol dipropionate, for inducing and maintaining lactation. 1 g. of an ointment containing 1% of this compound was applied locally to the udder of a virgin goat three times a week. A very small amount of milk was secreted daily for about 30 days, when the yield of milk of normal composition suddenly increased to 1·5 litres per day. This was followed by a slow decline as in an ordinary lactation. When the treatment was stopped during the lactation, the yield declined very rapidly, but was eventually restored by reapplication of the oestrogen. This work suggests that the administration of pituitary lactogens is not always necessary to induce actual secretion in the prepared mammary gland and also that oestrogens do not inhibit lactation in goats at the levels and under the conditions used by these workers. In more recent experiments with cows Folley *et al.* (95) have investigated the possibility

of using this same synthetic oestrogen for bringing about enrichment of the milk without an accompanying decrease in the yield such as was obtained in the previous experiments with naturally occurring oestrogens (93). Different methods of administration, such as feeding, injection, inunction and implantation, have been compared. The most promising results were obtained with implantation, for by this means the milk yield actually showed a slight increase of 0·7%, which was accompanied by an increase of 13·2% in the yield of fat and of 4·5% in the yield of total solids. The authors, as in a previous paper by Folley & Scott Watson (96), emphasize the dangers of excessive treatment with oestrogens which may result in abortion or in subsequent difficulty in getting the cow to conceive.

That stilboestrol has an inhibitory action on lactation under certain conditions has been shown recently by Noble (97). He has given stilboestrol to rats in their drinking water, beginning the treatment on the day following parturition. The growth of the litters was subnormal when the doses were small, but lactation was not entirely inhibited even by the largest amounts given. The subnormal lactation produced in this way was not improved by injections of pituitary preparations.

In the previous biennial review of this series (74) the work of Folley & Kon (98, 99) on the effect of sex hormones on lactation was cited. These authors found that the ability of a hormone to cause development of the mammae appeared to accompany the power to inhibit lactation. Thus oestrone, oestriol, oestradiol and testosterone were found to be inhibitors of lactation but also to cause growth of the mammary gland, whereas progesterone and androsterone did neither. Bacsich & Folley (100) have now published the results of a histological study of various glands in the normal and ovariectomized rats used by Folley & Kon. They found that the inhibitory action of the oestrogens was not accompanied by any marked involution of the mammary glands even where suckling had ceased sometime before autopsy.

Further work on the inhibitory action of the sex hormones has been published by Edelmann & Gaunt (101). They found that oestrone and pregnant mares' serum inhibit lactation in normal but not in ovariectomized rats. Some inhibition was also obtained with oestradiol propionate, diethyl-stilboestrol and testosterone propionate when given to castrated females, but the effect was less obvious in normal animals.

The clinical application of testosterone propionate for inhibiting lactation in humans has been described by Kurzrok & O'Connell (102) and by Beilly & Solomon (103). Three or four injections of 25 mg. were given at 12-hour intervals. Relief was obtained in some instances within 24 hr., and failure was observed in only 2% of the cases. The treatment did not appear to be accompanied by any discomfort nor were there any deleterious after-effects. Similar results have been obtained by Portes *et al.* (104) with testosterone acetate, while oestradiol benzoate has been used for the same purpose by Lehmann (105, 106) and by Foss & Phillips (107).

The apparent antagonism between oestrogens in inhibiting lactation and prolactin in promoting it has a parallelism in the pigeon-crop gland, a problem which has been investigated by Folley (108). On injecting oestrone along with prolactin preparations as much as 54% inhibition in the proliferation of the gland was obtained. Various theories which might account for this apparent antagonism are discussed. The fact that oestrogens do not appear to inhibit milk secretion by suppressing prolactin formation in the pituitary is suggested in experiments by Reece (109). He has estimated the prolactin content of the pituitaries of normal and spayed rats receiving injections of the commercially prepared oestrogen Progynon B. In the normal animals the prolactin content of the pituitary increased, while in the castrated animals hypertrophy of the pituitary was observed although the amount of prolactin in the gland was not altered. A somewhat similar study is reported by Reece & Mixner (110), in which mature spayed rats were treated with testosterone propionate which, as already

pointed out, is an excellent inhibitor of lactation. The treatment caused the prolactin content of the pituitaries to increase by about 40%, but the weights of the pituitaries remained unchanged. There is therefore no suppression of the prolactin formation in the pituitary to account for the power of this substance to inhibit lactation. A possible explanation for the fact that the lactogen secretion is increased and yet milk secretion inhibited by these compounds is suggested by Reece *et al.* (111), who have investigated the effect of a commercially prepared gonadotropic substance, Antuitrin S, on lactation in the rat. Connon (112) and other workers had previously found that this preparation was an inhibitor of lactation even when given in small amounts, whereas Hathaway *et al.* (113) were unable to obtain any such effect. Reece and his colleagues therefore reinvestigated the effect of Antuitrin S both when given alone and also when injected with Progynon B. They found that Antuitrin S alone did not significantly effect either lactation or the formation of lactogen by the pituitary, but that it did cause marked hypertrophy of the ovaries. When, however, it was given with the oestrogen, marked inhibition of lactation took place, although the weights of the pituitary glands and of the prolactin content of these glands were substantially increased. Microscopic examination of the mammae of the rats receiving both preparations showed many cells in mitosis, a condition which was comparatively rare in the other animals. It is suggested that this mitosis may account for the inhibition of lactation, since an actively proliferating gland is not likely to secrete so efficiently as one which is not proliferating. It will be of much interest to find whether further work will confirm this explanation of the inhibition which takes place with normal or even increased amounts of prolactin.

The effect of synthetic oestrogens on mammary gland proliferation has now been further investigated by Gomez & Turner (114, 115). A preparation of *p*-hydroxypropenyl-benzene (anol) has been found by them to stimulate development of both mammary duct and lobule-alveolar tissue in normal and castrated female rabbits and rats and in male mice. This preparation of anol therefore differed in its action from natural oestrogens, which in some species are believed to cause development of the ducts only and not of the lobule-alveolar tissue. The growth produced by this anol was found by the authors to be abnormal and atypical. The same workers (116) have used the method of Lewis & Turner (83) to estimate the production of Mammogen I by the pituitary under the influence of the anol.

The effect of a number of androgenic substances on the growth of the mammary glands of immature male guinea-pigs (117) and also on the weights of the testes and other glands (118) has been investigated in detail by Bottomley & Folley. One of the most interesting points arising from their results is that there appears to be a correlation between chemical constitution and the power to stimulate teat growth. Thus it was found that all the saturated androgens were almost, if not quite, inactive while all the unsaturated derivatives except one promoted definite teat growth. The presence of a double bond at Δ^4 or Δ^5 in the ring structure appeared to be essential if the substance were to possess marked activity. Another essential feature was evidently the presence of a hydroxy group on the 3 or 17 position, since testosterone was active and Δ^4 androstendione was not. It was also observed that dihydroxy derivatives appeared to be more active than the corresponding ketones. Van Heuverswyn *et al.* (119) have investigated the action of various androgens and oestrogens on mammary gland growth in male mice. Activity was shown not only by such widely differing substances as triphenylethylene, stilboestrol and dibenzanthrene but also by the hormone of the adrenal cortex, desoxy-corticosterone acetate. It is an extraordinary fact, particularly in view of the possible correlation between chemical constitution and the power to promote teat growth, that compounds which differ so markedly in their chemical composition and seem to have so little in common, should

all be capable of inducing mammary gland development. The fact that the hormone of the adrenal cortex possesses this property and that progesterone has now been isolated from the adrenal cortex by Beall & Reichstein (120) suggests that some of the results obtained in earlier experiments, in which the presence of the adrenal cortex factor had not been taken into consideration, may have to be re-interpreted.

The action of androgens on the mammary gland of the female rhesus monkey has been examined by Van Wagenen & Folley (121), who found that Δ^5-*trans*-dehydro*iso*androsterone, Δ^5-*trans*-androstendiol, *cis*-androsterone and testosterone propionate all failed to promote duct growth, although they caused duct dilation to varying degrees by inducing some secretory activity. Since large doses of testosterone propionate tended to cause a cystic condition of the ducts which did not occur with *cis*-androsterone, the authors suggest that the latter might be the more preferable of the two for clinical application in humans. An excellent paper on the mammary gland of the rhesus monkey under normal and experimental conditions has been published recently by Folley *et al.* (122). A detailed description, illustrated with exceedingly clear microphotographs, is given of the ducts and alveoli of normal glands and of glands which have come under the influence of oestrone and testosterone propionate. A paper on the same animal has also appeared recently by de Allende (123), who has studied the content of progesterone in the blood during the sexual cycle.

The effect of administering the hormone progesterone to spayed rats has been further investigated recently by Selye (124). He finds that the hormone is able to bring about a degree of development of the mammae in the ovariectomized animal similar to that which occurs in late pregnancy, and that to do this, no previous sensitization with oestrogens is necessary.

Thyroxine. The results of further experiments on the effect of thyroxine on milk secretion in cows and goats have been published during the last 2 years. Ralston *et al.* in a preliminary note (125) and later in a special bulletin (126) have described experiments in which 18 cows received a total of ninety injections of thyroxine. The injections of 15 mg. each were given on three successive days at monthly intervals throughout all stages of the lactation periods. Milk secretion increased till the sixth or seventh day after beginning the treatment and returned to normal 5 or 6 days later. The yields of milk, fat and non-fatty solids were increased on the average by 13·6, 22·5 and 14·7% respectively. It was found that cows in the second to ninth month of lactation were more responsive to the treatment than those at an earlier or later state and that animals at high production levels showed a greater increase in the actual pounds of milk and fat secreted but a smaller percentage increase than cows at a low level of production. Since prolonged hyperthyroidism in humans has in some instances shown deleterious effects on the cardio-vascular system, Ralston and his colleagues have made electro-cardiac records of goats and cows receiving thyroxine. Although the experiments showed that there was an increased heart output during thyroxine treatment no injurious effect on the heart has so far been observed with cows. With goats one out of the three animals tested showed indications of heart damage. Since it might be supposed that thyroxine would have deleterious results if given over abnormally long periods, it is important to note that Hurst *et al.* (127) have injected eleven cows with thyroxine for a period of 3 years and that there is no mention in the preliminary abstract of the paper to suggest that the treatment was at all harmful. It is recorded that milk yield and milk-fat production during this period showed increases of 30 and 59%. Apparently there was a possibility both in this work and in that of Ralston *et al.* (126) that seasonal variation affects the thyroid-mammary relationship. The full details of Hurst's experiments are, however, not yet available.

The effect of thyroxine on the blood lipoids and on the nature of the milk fat of the cow has been studied in detail by Smith & Dastur (128). The usual marked increase in milk yield took place during thyroxine treatment, but there was no alteration of any significance in the nature of the milk fat except for a slight temporary change at the beginning of the hyperthyroidism period when the gland was becoming accustomed to its increased production. This lack of alteration in the nature of the fat during the period of enhanced yield suggests that an increased production of milk and milk fat brought about by administering thyroxine or by breeding cattle with larger or more active thyroids would not be likely to result in a butterfat of inferior composition or diminished keeping qualities. Smith & Dastur found that the concentration of sugar in the blood plasma increased by some 10–26% during the treatment and that this was accompanied by a decrease of 10–20% in the concentration of the various plasma lipoids. The level of non-fatty solids in the milk during thyroxine treatment showed little alteration. There seemed to be a tendency for the lactose content to increase and the protein content to diminish, but these changes were slight.

In the previous review (74) it was pointed out that two groups of workers were not agreed as to the effect of thyroidectomy on lactation. Nelson & Tobin (129) had found that removal of the thyroid gland in rats did not adversely affect lactation, whereas experiments described by Folley (130) led to the opposite conclusion. Nelson (131) has now published further details in which he confirms his earlier observations. In his later experiments he thyroidectomized rats during pregnancy and during lactation and recorded the effect on the weights of the young. After 21 days the average weight of the young from normal mothers was 35·3 g., from those thyroidectomized during pregnancy 35·7 g., from those treated during lactation 31·8 g. and from those thyroidectomized in a previous pregnancy or lactation 36·3 g. The results suggest that milk secretion was slightly inhibited only with those animals from which the thyroid had been removed during that particular lactation. In view of the well-known influence of thyroxine on milk secretion it is difficult to explain Nelson's findings, which are so much at variance with those of Folley.

The time is rapidly approaching when hormone therapy applied with the object of improving milk secretion will become available for practical use. One of the factors which has always tended to be prohibitive in this connexion is the expense of the various preparations and the fact that they have usually to be administered by injection. So far as thyroxine is concerned this problem is now approaching a solution, for in recent years Ludwig & Mutzenbacher (132) made the discovery that a substance having thyroid activity could readily be made by treating casein with iodine under certain prescribed conditions, a fact which was later confirmed by Harrington & Pitt Rivers (133). Turner (134) has now given a preliminary report of experiments in which this active substance, which he terms "thyrolactin", has been prepared by adding powdered iodine to skimmed milk and precipitating the protein at the isoelectric point, after which the iodinated casein is dried and ground. The substance so formed has the advantages that it is cheap to prepare and readily incorporated in the feed of the animals. Turner expresses the hope that workers at various centres will prepare this thyrolactin and test its usefulness in practice. Should it ever prove of practical value a method of assaying its thyroid activity will be essential. This point has been investigated by Heathman & Turner (135), who have been experimenting with a method based on the inhibition of growth brought about in day-old cockerels as a result of thyroxine or thyrolactin feeding.

Adrenalin. The effect on blood sugar and on milk secretion of implanting adrenalin tablets subcutaneously in goats and cows has been studied by Bottomley *et al.* (136). By this means they were able to obtain a state of hyperglycaemia which in some

instances lasted for 42 hr. and was accompanied by an increase in the lactic acid content of the blood. As a result of the treatment the percentage of lactose in the milk was slightly raised, but in none of the experiments was the milk yield increased. In fact, in three instances the yield was actually diminished, probably as a result of trauma associated with the operation. No marked or uniform effects were observed for the milk fat, but the non-fatty solids content appeared to rise slightly and temporarily.

The role of the posterior pituitary in lactation. Gomez (137, 138) has further investigated the part played by pituitrin in lactation. Hypophysectomized lactating rats were given daily injections of adrenal cortical extract, glucose and lactogenic anterior pituitary preparations. The young of these rats died in 2–3 days due to their inability to obtain milk, although they suckled the glands which contained it. On injecting the mothers with pituitrin, however, the young survived for 28 days or more, but lost weight as soon as the pituitrin administration was stopped. This necessity for posterior pituitary extracts for the ejection of milk from the mammae of cows has also been studied by Ely & Petersen (139). They have shown that ejection of the milk as distinct from secretion is favoured by the presence of posterior-lobe hormones in the blood and inhibited by adrenalin, and that the amounts of these substances in the blood are controlled by afferent stimuli which reach the central nervous system from various sources. Thus fright or administration of adrenalin prevents ejection of the milk, a process which is once more resumed on administering pituitrin. The authors present evidence which suggests that the posterior lobe hormones act by causing the alveoli and ductule musculature to contract and thus eject the milk.

Biochemical aspects

One method of approach to the study of milk secretion which has been extensively investigated during the past 10 years depends on the principle of obtaining representative samples of the blood entering and leaving the actively secreting mammary gland with the object of determining the nature of the milk precursors and the amounts in which they are used by the gland. The most recent work on this subject has been carried out during the past 2 years at two research stations in America, one at Missouri led by Turner and the other at Minnesota led by Shaw & Petersen. Both these groups of workers realize that if the arterio-venous differences recorded in experiments of this type are to be reliable the animals must be subjected to the minimum amount of disturbance and excitement before and during the time of sampling, a fact which was also emphasized a few years ago by Graham *et al.* (140). Partly in an endeavour to overcome this difficulty Petersen *et al.* (141), whose general views have been briefly summarized in an article by Petersen & Shaw (142), have elaborated a technique in which the mammary gland is excised and the excised gland perfused with blood, the essential parts of the apparatus consisting of an artificial heart, a resistance for maintaining constant blood pressure, a bath for controlling the blood temperature and an oxygenator. The authors claim that the perfused excised gland functions normally since blood sugar, fat and nitrogenous substances are all taken up by the gland in amounts which correspond with those of the intact gland. Some of the advantages of the procedure are stated by the authors to be that many variable external factors are eliminated while the rate of blood flow and blood pressure can be controlled and measured. Moreover, an investigation can also be made of the effect of increased and decreased amounts of precursors in the circulating blood, while the lymph can also be studied. On the other hand Reineke (143), from the Missouri station, while agreeing that perfusion technique may certainly eliminate some of the variable factors encountered in the intact animal, suggests that

the procedure is open to the objection that the storage depots of the body are eliminated from the system, with the result that the circulating blood is not truly arterial after one or more circulations and that some of the endocrine factors concerned in lactation may not function normally in such a system. Reineke has therefore developed a technique in which the animal is completely anaesthetized with nembutal before the arterial and venous samples are taken. He believes that in this way, while the milk secretion processes function normally, the animal is immune from excitement and other undesirable disturbances.

These two different methods, perfusion and anaesthesia, have been applied to the determination of the respiratory quotient of the mammary gland. As pointed out in the previous review Graham *et al.* (144) found, from a large series of arterial-venous samples obtained from lactating goats, a mean R.Q. of 1·36. Since this R.Q. was greater than unity, it was interpreted in the classical way as indicating that in the mammary gland a portion of the milk fat is synthesized from other precursors. This theory gained support from the observation that the amount of milk fat secreted could not be entirely accounted for by the amount of blood fat removed by the gland. Shaw (145) has now put forward evidence against these findings on the grounds that during arterial and venous sampling of normal animals there may be very considerable changes in blood volume which will substantially affect the arterio-venous differences and the conclusions drawn from them. The fact that these large volume changes occur was shown by Shaw & Petersen (146) and also by Petersen & Boyd (147) by means of haemoglobin analysis. In twenty-three experiments in which the cows were not excited, blood volume changes during sampling exceeded 1% in only three instances, whereas in a similar number of experiments in which the animals were obviously excited, volume changes took place in every instance and varied from +1·5 to −14·5%. On attempting to correct the arterio-venous differences for these volume changes it was found that the values obtained for the uptake of glucose were fairly uniform whether volume changes occurred or not, but for fat and protein reasonable values were unobtainable where volume changes had occurred. The authors explain this finding by suggesting that when appreciable amounts of fluid pass to and fro between the blood and the mammary gland as a result of excitement before or during sampling, considerable quantities of the non-diffusible blood constituents such as fat and protein accompany the fluid. It therefore becomes impossible to interpret the arterio-venous differences for these non-diffusible constituents when the animals have been unduly disturbed. The fact that such volume changes would also be likely to affect the R.Q. led Shaw to measure the R.Q. for disturbed and undisturbed intact animals and for the perfused excised mammary gland. He found that in the intact gland when no blood volume changes took place the R.Q. varied from 0·93 to 1·70. When, however, blood-volume changes were observed the uncorrected values for R.Q. varied between 0·56 and 2·26 and became 0·22–0·99 when corrected. For the non-lactating gland of the intact animal a value of 0·2 or less was obtained. These values for the intact animal are extremely difficult to interpret. In perfusion experiments, however, the R.Q. of the dry gland was approximately 1·0 and for the lactating gland it was 0·8. Shaw assumes therefore that the values obtained for the perfused lactating gland, where the R.Q. was 0·8, are the correct ones and that in the lactating gland fat is oxidized and not synthesized. In his anaesthesia experiments, however, Reineke (143) supports the original findings of Graham *et al.* (144) and claims to show that the R.Q. is greater than unity. Thus for completely anaesthetized goats he obtained a mean value of 1·09 with a mean deviation of 0·06. When only the artery and vein were anaesthetized the corresponding values were 1·15 and 0·15, while without anaesthesia of any sort they were 1·17 and 0·19. Reineke points out that the variation is least in the completely

anaesthetized animal and that in each instance the value is greater than unity. From this fact, and also from a comparison of the ratio of glucose plus lactic acid uptake to the uptake of fat with the ratio of lactose to fat in the milk, Reineke concludes that the amount of blood fat removed by the gland was not sufficient to account for the milk fat produced and that therefore fat is synthesized in the lactating gland. It seems worth while observing, however, that from Reineke's own claims the nearer he gets to the ideal conditions for arterio-venous sampling, the nearer does the R.Q. approach unity. In another paper from the same station (148) it is again stated that the R.Q. of the non-lactating gland in the intact goat is less than for that of the lactating animal. It is hoped that in the near future further work will explain the discrepancy between these opposing views.

In the paper just cited Houchin *et al.* (148) record a large number of analytical results for various constituents of arterial and venous blood and plasma from lactating and non-lactating goats. The authors hope that these will act as normal standards for future work on simultaneous arterio-venous sampling.

With regard to the secretion of milk fat a number of publications have recently appeared in addition to those which have already been discussed and which deal with respiratory quotients. Kelly & Petersen (149) have carried out experiments to discover the actual site of milk-fat production in the gland. They first fed stained fat to cows and studied the fats in the gland and in the milk. Owing to the destruction or dilution of the dye these experiments were, however, of little value. They next determined the acid value of the fats extracted from the glands at different degrees of development and activity with the following results. When the glands had not undergone the development which occurs during pregnancy, the acid value was as low as 0·7, for dry glands which had previously lactated it was 13–23, for actively lactating glands it was 38 and for one gland at the end of lactation it was 19. It therefore appears that the amount of free fatty acid in the gland is related to the activity and development of the gland. By staining slices of epithelial tissue with three different dyes known to distinguish between neutral fat and free fatty acids, it was found that the free fatty acids were distributed throughout the epithelial tissue, but that they were more concentrated in the basal portions of the alveolar cells than at the distal ends, where neutral fat globules were more numerous. The authors believe that the free fatty acid is in a molecular or colloidal state at the basal end and that on being converted to neutral fat there is a change in interfacial tension causing the particles to coalesce and become of normal milk-particle size at the lumen.

The formation of the lower fatty acids in milk fat is still a problem which remains unelucidated. As explained in the previous review (74) Hilditch and his co-workers have supplied valuable evidence to suggest that these lower members arise in the mammary gland by oxidation and subsequent reduction of oleyl groups which are present in the form of oleyl glycerides. This view has been supported recently by the work of Shaw (145), who finds that sufficient blood fat is taken up by the gland to account for the total milk fat produced and who believes from R.Q. measurements that fat is not synthesized but oxidized in the gland. Further evidence which might be taken as confirming Hilditch's theory has also been obtained by Smith & Dastur (150) in experiments on the effect of inanition on the composition of milk fat. Although no appreciable change was detected in the nature of the lipoids of the blood plasma on inanition, as judged by their general characteristics, the nature of the milk fat underwent a substantial change. A decrease of about 80% occurred in the content of lower acids up to and including myristic, and this deficiency was almost entirely made good by an increase in the content of oleic acid. There seemed, therefore, to be a close inverse relationship between the contents of oleic acid on the one hand

and of the lower fatty acids on the other, which could readily be explained by the theory that the lower members are formed by oxidation of oleic acid. There are, however, three points which still require to be reconciled with this theory: first, the R.Q. of the mammary gland as found by the Missouri workers is always greater than unity and thus suggests fat synthesis rather than breakdown; secondly, it has been found at Missouri that the amount of blood fat taken up by the gland is insufficient for the total milk fat produced and that breakdown of fat is therefore unlikely (these two points are both disputed by the Minnesota workers); thirdly, it has been shown at Minnesota that the actively lactating gland contains free fatty acids. It would seem strange that in the presence of such free fatty acids the gland should have to obtain energy by the circuitous route of oxidizing oleyl glycerides at the alkyl end of the fatty acid chains followed by reduction of the resulting products, when direct oxidation of the free fatty acids might presumably be more readily accomplished. Ultimate proof for this attractive theory of Hilditch is therefore still required.

The blood fat of dairy cattle has been studied by Allen (151), who has devised a volumetric method for measuring "blood fat" by a process similar to that used for estimating the fat in milk. At the most only 3 ml. of plasma are required for each determination and 100 samples of plasma can be collected and tested in 3 days. As little as 0·25 ml. of plasma can be used if desired. The value for "blood fat" measured by this means includes sterols, sterol esters, neutral fat and fat-soluble pigments, but not phosphatides. Allen has used this method to study the effect of various factors such as age, breed, sex, gestation, lactation and season on the "blood fat" in a herd of over 100 dairy cattle of four different breeds. It is impossible in this brief review to enumerate the large number of interesting conclusions at which Allen arrives, but one or two of the more important may be mentioned. He found that the blood plasma was low in fat immediately following parturition, rising rapidly to reach a maximum after about 4 months. From then onwards there was a gradual decline to a low level during the dry period. By suitable feeding in the dry period it was possible to increase the blood-fat level to the highest values existing during active lactation, which indicates that fat intake is an important factor in determining blood-fat level. At the same time, however, Allen points out that lactation probably exerts an influence on this level independent of the amount of fat ingested. Although the cows showed a marked individuality in the fat content of their plasma, no systematic relationship was found between the fat content of the blood plasma on the one hand and the yield and percentage of milk fat on the other. The author does not believe that the fat content of the blood plasma alone is of practical value as an indication of producing ability or as a criterion for selection for breeding purposes. The fact that other factors may be more important than blood-fat level has been shown by Smith & Dastur (128), who have found that the increase of 30 or 40% in daily fat yield which is so readily brought about by thyroxine administration is accompanied by a decrease of 10–20% in the level of the total lipoids of the blood plasma and of their main components.

Further papers have been published recently on the actual nature of milk fat and its component acids. Hilditch & Jasperson (152) have studied in detail the octadecadienoic components. Although it is certain from earlier work by Hilditch and his colleagues that an acid of this type is present in milk fat no conclusive evidence has yet been obtained as to its precise structure. Hilditch & Jasperson have shown that neither the natural *cis*-Δ^9-*cis*-Δ^{12}-linoleic acid found in seed fats nor the *trans*-Δ^9-*trans*-Δ^{12}-isomer is present to any appreciable extent, but it is still unsettled as to whether either of the other two geometrical forms of this compound is present. The view that the linoleic acid of butterfat is a different isomer from that in seed fat has also been suggested by the work of Houston *et al.* (153), who found that the

most unsaturated fractions of butterfat show marked spectroscopic absorption at 230μ which does not occur with the linoleic acid of seed fat or with that contained in grass. Hilditch *et al.*(154) have also made an extensive study of the component glycerides of a typical milk fat secreted by cows at summer pasture. Owing to the large number of individual fatty acid groups present such a problem is no light task, but it has now been accomplished by fractional crystallization of the fat from acetone followed by a detailed analysis of the three resulting fractions. The results of this extensive investigation suggest that the triglycerides of butterfat are very similar to those of ox depot fat except that the stearyl radical in a proportion of the stearo-glycerides of the depot fat have been partly replaced in butter by lower fatty acid radicals. The authors point out that this finding supplies further evidence, though not proof, for the theory previously put forward by Hilditch that the stearic groups of the depot fat and both the stearic and lower fatty acid groups of the milk fat may be derived from preformed oleoglycerides. Further evidence of a similar nature is discussed briefly by Dhingra & Haneef(155), who have analysed the body fat of female goats. It will be well to stress at this point, however, that if any theory for the formation of milk fat is to be based, no matter how slightly, on apparent similarities between the glycerides of milk fat and those of depot fat, the presence of free fatty acids in the mammary gland during active lactation, to which reference has already been made, will have to be taken into consideration. Analysis of butter glycerides is also being carried out by Meigs(156), who states that he is developing a method for separating the saturated and unsaturated esters by the addition at the double bonds of thioglycollic and thioacetic acids. He reports the isolation of a glyceride of stearic, palmitic and myristic acids. In experiments on the hydrolysis of butterfat by pancreatic lipase it has been shown by Peltola(157) that butyric and hexanoic acids are liberated more rapidly than oleic in the earlier stages of hydrolysis, but that after 40% of the fat has been changed all three acids are liberated at the same rate.

The phosphatides of milk have been investigated by various authors. Weckel *et al.*(158) have found that the content of phosphatides, unlike that of the milk fat, is highest in the foremilk and lowest in the strippings. The fatty acids of the sphingomyelin-cerebroside fraction have been isolated and analysed by Kurtz & Holm(159). This fraction, isolated from buttermilk powder and found to be insoluble in both acetone and ether, appeared to consist of approximately equal parts of sphingomyelin and cerebrosides. Fractionation of the fatty acids as methyl esters showed that about 80% of the acids were made up of the typical lignoceric acid mixture and that the remaining 20% was a mixture of various saturated, unsaturated and hydroxy acids. The sphingomyelin-cerebroside fraction of milk is therefore probably similar to that in other tissues.

Studies on the blood precursors of milk protein depending on the arterio-venous technique have now been fully reported in a bulletin by Reineke *et al.*(160). The results confirm the observations previously made by both the Missouri and Minnesota workers(161, 162) that the amino-acids absorbed by the mammary gland are insufficient to account for the total nitrogen of the milk. Other blood constituents which may be involved are globulin, albumin, fibrinogen and some non-protein nitrogen factor other than urea. From the work of Shaw & Petersen(162) it is unlikely that this last factor can be uric acid, creatine or creatinine. It was found by Reineke and his colleagues that as blood passed through the mammary gland there were marked changes in the partition of the plasma protein into fibrin, albumin and three globulin fractions. The changes seemed to the authors to be large enough to exceed experimental error. They point out, however, that the venous blood is some 6·5 volumes % higher in CO_2 than arterial and that the effect of this on the protein partition is at present unknown. The most consistent observations with regard to the exchange of

plasma proteins between the blood and the mammary gland were those for fibrinogen. There was an uptake of this protein for the first 70 days of lactation in goats, after which it was apparently given up to the blood by the gland. The authors put forward the suggestion that a series of reversible changes may possibly occur in the mammary gland by which albumin, globulin and fibrinogen became interconvertible and by which a portion of their molecules can be used for casein synthesis. In a later paper by Reineke(143) it is stated that analysis of the carbohydrate portion of the plasma proteins indicates that carbohydrate material is taken up by the gland in significant amounts as a glycoprotein complex. Such a substance might serve as a precursor of both sugar and protein and also of fat, if fat synthesis is really one of the functions of the lactating gland. It may be worth while to point out here that an unidentified milk constituent which may well have been a glycoprotein complex was detected by Smith *et al.*(163) in the milk secreted by cows during inanition. It constituted about 2% of the milk or 20% of the total milk solids.

Further work on the proteins which are associated with milk secretion has been carried out recently by Jackson & Gortner(164), who have examined the properties of the proteins obtained from the active and inactive glands. The only significant difference between the proteins of the active and inactive tissue appeared to lie in the albumin-globulin ratio, which was higher in the active gland. The nitrogen distribution of various protein fractions isolated from glands could not be correlated with the degree of glandular activity, nor were there any marked differences in amino-acid composition. The amino-acid composition of the proteins from human and cows' milk has been investigated by Beach *et al.*(165). Cows' milk contained 405 mg. of casein nitrogen and 89 mg. soluble protein nitrogen per 100 g. The corresponding values for human milk were 49 and 77. The amino-acid distribution in the two milks is recorded for histidine, arginine, lysine, tyrosine and tryptophane. Judging from these five components, which were present in both milks in much the same relative proportions, there should be no significant differences in nutritive value between cow and human milk protein.

There is little work to report in the present review regarding the synthesis of lactose by the mammary gland, but one paper by Daoud(166) is of particular interest in illustrating how even with the abnormally high glucose levels existing in the blood of diabetic lactating women no glucose passes into the milk, nor does the concentration of lactose in the milk increase during severe hyperglycaemia. The ability of the mammary gland to prevent glucose from passing into the milk under these conditions is particularly noteworthy.

Anatomical aspects

A most valuable mimeographed volume on the comparative anatomy of the mammary gland has been published recently by Turner(167), whose own researches have accomplished much in this particular field. The volume is most efficiently produced with excellent illustrations and should prove an indispensable addition to the library of all those who are interested in lactation whether in the human species or in farm animals.

Since dairy cows are judged so much by their outward conformation, Swett & Graves(168) have subjected this procedure to a very stringent test by studying the relationship between conformation on the one hand and internal anatomy on the other. A statistical analysis is given of records collected for more than 350 cows. 169 correlations have been worked out and show the relation of heart weight, lung weight, intestine length and live weight to other ante- and post-mortem items and also to each other. There is a lengthy summary to the paper in which a large number of conclusions are discussed. One typical example which may be cited from this

summary is the statement that in general live weight is rather highly correlated and heart weight moderately correlated with body dimensions and anatomy in cows, while lung weight and intestine length appear to have comparatively little relation to body dimensions. The relation of conformation and anatomy to producing capacity has also been studied by Graves (169). He found that the capacities for 157 lactating udders averaged 53·2 lb. compared with 32·0 lb. for 104 non-lactating udders. Capacities of calves' udders increased steadily from 0·06 lb. at under 3 months to 6·64 lb. at 12–18 months. For ninety-four cows the left and right rear quarters produced 29·2 and 29·0% of the total milk yield respectively, while the left front quarter produced 20·2% and the right front quarter 21·6%. In 185 udders the average capacity of the front quarters was 4880 ml. and of the rear quarters 5666 ml. Even greater differences in the same direction were noted in the depth of glandular tissue. Work on the conformation of the red dairy cattle of Southern Bessarabia has been carried out by Mauch (170). Correlations are given between such factors as milk yield on the one hand and fat content, total fat yield and length of lactation on the other. There was no significant correlation between milk yield and the structure of the hair or the intensity of its pigmentation. The micro-structure of the mammary gland of the small Caucasian breed has been examined for thirty-eight cows by Arsoumanian (171). The relative proportions of glandular connective and fatty tissue in the udder were studied throughout lactation. It was found that the proportion of glandular tissue diminished from 70% during the first 5 months of lactation to 56% in later lactation and 48% in the dry state, the corresponding values for connective tissue were 22, 33 and 35%.

Clinical chemistry

Variations in the blood content of magnesium, calcium and phosphorus have so frequently been associated with pathological conditions or with abnormal conditions of feeding and management that the level of these elements in blood has for long been regarded as of first class importance. In consequence much work has been published recently on this branch of the subject. Most of the papers which have appeared in the past 2 years have described experiments in which the main object has been to establish reliable standard values for the level of these three constituents in the blood of normal well-nourished dairy stock, in order that the standards so obtained may be available for comparison with the values recorded for animals known to be existing under abnormal circumstances or believed to be in a pathological condition.

A series of papers on this subject is now being published by Duncan and his colleagues. The first two (172, 173) deal with the magnesium content of the blood plasma of young dairy cattle. Magnesium was estimated for 107 normal calves at intervals of 1 or 2 weeks over a period of 3 years. The mean value tended to increase from $2{\cdot}389 \pm 0{\cdot}015$ at birth to $2{\cdot}591 \pm 0{\cdot}032$ mg./100 ml. at 1 year, and this increase was accompanied by a series of rhythmic variations lasting for several months. The mean of 2286 determinations was 2·41 mg./100 ml. with a range of 1·62–3·83. The authors have shown by statistical analysis that the range of normal variation is probably sufficiently wide to include many variations that occur in pathological conditions, so that wide fluctuations from the average cannot in themselves be regarded as signs of abnormality. The numerous results have also been statistically analysed without reference to the age of the animals but with respect to the month and season of the year. There was found to be a distinct parallelism between seasonal changes and the magnesium level in the blood. The value remained fairly constant from November to April, but in May and June, during the change from winter to summer conditions, a rapid decline was observed, and this was followed by an increase from July to

November. Various climatic factors, such as hours of sunshine, temperature, humidity and barometric pressure, were considered, but although some relationships were found between these various factors and magnesium level not one of them accounted entirely for the various changes which were observed. Lower magnesium values seemed to be associated with months in which there were more than 8 hr. of sunshine and in which the mean and maximum temperatures were above 55 and 70° F. It appeared, however, that solar radiation alone could not account for this difference, since calves kept away from direct sunlight responded in the same way during this particular season. The authors wisely point out the importance of carrying out magnesium estimations under highly standardized conditions, since very slight differences in procedure and in the method of treating the blood sample before analysis can materially affect the result. Allcroft & Green (174) have also estimated magnesium in the blood of Hereford cattle kept throughout the year at pasture. They observed a maximum value in August, which fell to a minimum in December, the extreme range being 2·8–0·5 mg./100 ml. The lower values, which could be raised to some extent by feeding magnesium oxide mineral mixtures, were not accompanied by any clinical symptoms. An instance of hypermagnesemia without clinical symptoms is reported by Eveleth *et al.* (175), who observed this condition during a drought when the animals were at pasture. The mean values for forty-eight cows at different times of the year were January 2·81, April 2·95, July 4·01 (when the rainfall was very low), and September 2·91 mg./100 ml. There was also found to be an inverse relationship between magnesium level and rainfall in the work of Duncan *et al.* (173). It is of interest to note that Eveleth and his co-workers found that the calcium and magnesium varied independently of one another. Wallis (176) has studied the blood magnesium of dairy cattle in relation to vitamin D deficiency. No consistent changes were found which could be attributed to the development of this deficiency even in those instances where the calcium and phosphorus of the blood were decidedly subnormal.

The concentration of blood inorganic phosphorus has been investigated in some detail by Johnson (177). He has determined the level of this constituent in the blood of 200 cows and heifers in Pennsylvania during the spring, when the animals had not been to pasture for 4 months. In heifers the value of 6·42 mg./100 ml. at 12 months fell to 4·70 mg. at 30 months, the average for sixty-four milking cows being 4·33 mg. By statistical analysis no difference could be found between 2–3- and 4–5-year-old cows, but older animals tended to have lower levels than those of medium age. For cows kept under optimal conditions of feed and management there were no differences in blood inorganic phosphorus between lactating and non-lactating animals, nor did variations in the daily milk yield affect the level. These findings should be considered along with those of Van Landingham *et al.* (178, 179), which were cited in the previous review and with which they are not entirely in agreement. The latter authors record, among several other important observations, that in their experiments there was a decrease in the inorganic phosphorus level from 4·95 mg. in the first lactation, through 4·81 mg. in the second to 4·35 mg. in the third.

The effect of gestation and lactation on the blood levels of calcium, phosphorus, glucose and non-protein nitrogen has been investigated by Kennedy *et al.* (180). It was found that the values for these constituents during pregnancy did not differ significantly from those during lactation. An interesting observation made by the authors is that the mean blood sugar level for calves of less than a week old was 125 mg./100 ml. and that this figure gradually declined to 54 mg. by the end of the first year. Reichert (181) has also published the results of a very extensive analysis of cows' blood. The constituents which he has determined in the jugular blood of sixty lactating and twenty dry cows, all normal, include sugar, N.P.N., urea, uric

acid, creatinine, cholesterol, Na, K, Ca, Mg, Cl and P. He found that the Ca/P ratio was 2·46 in heifers, 2·15 in pregnant cows and 2·04 in lactating cows. The effect of milking on various constituents is discussed.

An extensive study has recently been made of the partition of serum calcium in the blood of Ayrshire cows. Since a variety of pathological conditions in the cow is associated with hypocalcaemia it is important that as much as possible should be known about the various forms in which this element exists in blood. To establish standards for the different forms Godden & Duckworth (182) have carried out over a period of 3½ years estimations on fourteen cows each of which had completed one pregnancy. The detailed results are too numerous for brief discussion, but a few of the principal findings may be mentioned. It was found that the total serum calcium remained fairly constant at an average of 10·4–10·6 mg./100 ml. for the first 12 or 14 months, after which it increased and ultimately attained a value of 11·7 and 12·4 mg. after 3 and 3½ years. The way in which this total was accounted for by the different fractions and how these fractions varied during the 3½ years may be stated approximately as follows. The calcium ion fraction (10–12%) remained remarkably constant throughout. The ultrafilterable calcium complex (40–45%) remained at 4·4 mg. initially and then rose finally to 5·3 mg. The non-ultrafilterable complex (20%) remained at 2·2 mg. for the first 3 years and then rose to 2·8. The protein-bound calcium (25%) increased slightly from the original value of 2·8 to 3·1 mg. at the end. The fractions which are regarded as "physiologically available" calcium are the calcium ion and the ultrafilterable calcium complex. These together amounted to 50–53% of the total throughout the 3½ years. Duckworth & Godden (183) have also investigated the changes in these calcium fractions before and after parturition. They found that all the values for total calcium were higher a few days before calving than those observed either a few weeks before or after. At the actual time of parturition there was a general reduction of about 10% in the total value. The ultrafilterable and non-ultrafilterable calcium complexes always showed a reduction at parturition, while the calcium ion fraction and protein-bound calcium were usually but not always greatest at this period. Incidentally it was observed that the level of the non-ultrafilterable calcium complex generally behaved similarly to that of the inorganic phosphorus, a fact which was also noted by Ray (184). This latter author studied the effects of a change from a phosphorus-deficient to a phosphorus-adequate diet and also of inanition on the calcium partition in ruminants. In both instances there was an increase in serum inorganic phosphorus and a reduction in total serum calcium, the non-ultrafilterable calcium complex increasing with the inorganic phosphorus while the other fractions tended to diminish.

The partition of the serum globulins of the dairy goat by sodium sulphate at various concentrations has been recorded by Reineke *et al.* (185). These authors have shown that the serum globulin can be partitioned into euglobulin, pseudoglobulin I and pseudoglobulin II and that the latter two fractions can probably also be subdivided.

Climatic and other factors affecting milk secretion

A knowledge of the effect of climatic factors on lactation is of much potential value in at least two directions. Firstly, it may throw light on the nature of some of the physiological processes involved in milk secretion, and secondly, it may serve as a guide to the best conditions of management for herds in different parts of the world throughout the changing seasons of the year.

One of the most important factors which has been investigated by workers in the past is temperature. This has now been studied under excellent conditions in California by Reagan & Richardson (186), who kept cows in a large room in which the

temperature could be varied from 40 to 100° F. while the relative humidity was maintained at 60% and the air movement at 60 cu. ft./min. It was found that as the temperature increased over this range the pulse rate of the cows decreased from 72 to 57 beats per min. At the same time the respiration rate closely followed Van 't Hoff's law for chemical reactions and was approximately doubled for every increase of 18° F., rising from 12 to 124 respirations per min. between 40 and 100° F. Since the authors were able to show that the cow does not sweat to any measurable extent, and since its hide is such a bad conductor of heat, it was concluded that the principal method by which the animal maintains its body temperature under differing external conditions is by controlling its rate of respiration. The rectal temperature of the cows remained almost constant at 101–101·3° F. when the room temperature varied between 40 and 70° F., but above that point the rectal temperature steadily increased until it attained 105·1° F. at an external temperature of 100° F. Until the hyperthermic stage was reached, between 70 and 80° F., there was little or no effect on milk secretion, but beyond that temperature the yield of both milk and fat diminished rapidly. The content in the milk of non-fatty solids also decreased from 8·26 to 7·58% and the casein from 2·26 to 1·81%. Up to 95° F. the actual composition of the butter fat, as judged by Reichert Meissl and iodine values, appeared to remain fairly constant, but above that temperature it tended to become more unsaturated and to contain less of the lower fatty acids. Perhaps, at the higher temperatures, the usual milk precursors were not supplied to the mammary gland either at the same level in the blood or at the same rate. This suggestion gains at least partial support from the diminished pulse rate observed at the higher temperatures and also from further data published by Reagan & Mead(187), which shows that above 80° F., when the cows had been in a hyperthermic condition for 24 hr., water consumption and food intake were both diminished. The subject has also been investigated in the North Dakota region of America by Dice(188), who began his experiments with the object of showing the folly of turning cows out of the byre during the day in extremely cold weather. From the results of his work, which extended over four winters, the author concluded that even in the coldest weather a warm byre is an advantage only to those who have to attend to the cows and not to the cows themselves! His results show that, provided the animals have adequate rations, shelter from wind and snow, and dry bedding, they will produce the same amount of milk of the same quality when exposed to extremely low temperatures as they do when housed in a warm byre at 50° F. Moreover when exposed to the cold, they will actually put on more weight although receiving the same rations. From the experiments of Reagan & Richardson just cited, it may well be that at very cold temperatures, due to her non-conducting hide and reduced respiration rate, the cow is able to conserve heat to a remarkable degree, with the result that on the same rations in the colder conditions there are at least as much nutrient material and calories available for storage and for milk production as there are at higher temperatures. The increased pulse rate at lower temperatures might also lead to the mammary gland receiving in a given time a greater supply of the milk precursors than under corresponding conditions at higher temperatures when the pulse rate is diminished.

Several other papers have appeared recently which describe climatic effects, but which tend to deal not so much with only one specific factor as with the general seasonal trend throughout the year. This may naturally involve many factors, such as seasonal changes of feed and management. It will only be possible in the present review to deal very briefly with most of these publications. In view of the work just cited by Reagan and his colleagues and by Dice, it is interesting to note that one or two workers in different parts of the world have added to observations previously

made that there is a tendency for milk composition to be inferior in the summer months, particularly if the conditions are unusually hot or dry. This fact has been discussed recently both by Trambics (189) in Germany and Jacobsen & Wallis (190) in South Dakota. The latter workers made 1-day tests of milk composition every month over a period of 5 years for cows of four different breeds. They found that low values for several of the milk constituents occurred in the summer, especially in July, and also incidentally in early and late lactation. In addition they studied in some detail the alterations in composition as colostrum changes to normal milk. Caulfield *et al.* (191) have carried out a somewhat similar experiment over one complete lactation period in Kansas. The variations in milk composition for four different breeds from month to month are recorded. The fat percentage appeared to be lowest in July and August, but the level of non-fatty solids remained between 8·9 and 9·2% throughout the whole period except for a drought in July 1937, when it fell to 8·0. This low value was not repeated in the following July when the rainfall and temperature, and therefore the pasture, were normal.

The general effect of season on the actual composition of butterfat has been discussed in the past 2 years by a number of workers. In Holland (192, 193) a study of the Reichert Meissl (R.M.) values of over 16,000 samples has shown that low values occur mainly during the months of July to December, and that an inverse relationship exists between the refractometer number of the fat and its R.M. value. Platon (194) in Sweden has observed a fairly close direct correlation between the iodine value and refractometer reading of the fat. The latter varied from 39·5 to 43 at 40° C. for a range in iodine value of 24·5–36·5. There was a tendency for low iodine values to occur in the autumn and winter months. These findings fit in well with the observation which has been made on a number of occasions that the R.M. and iodine values of butterfat frequently tend to vary inversely. That this is not always so has, however, been shown by Stout & Stein (195) working in Oregon. They observed that the R.M., refractometer and iodine numbers all reached a maximum in August and decreased to a minimum in December, rising again to some extent by April. Davis & Griffiths (196) also found for Danish and Welsh butterfat that the R.M. and iodine value were both highest in the summer. Doubtless in these instances the type and quality of the grazing are factors of as much importance as the climatic conditions themselves. This is borne out by French (197), who found that in Tanganyika maximum values for the R.M. and iodine value of 28 and 34 respectively occurred in February and changed to corresponding figures of 25 and 29 in June, the alteration in the composition of the fat accompanying the change-over from young grass to mature dry roughage. Das Gupta (198) also observed significant increases in R.M. during November to February, when grass was fed, as compared with the values from July to October, when the grass was replaced by paddy straw. The iodine value tended generally but not invariable to alter inversely with the R.M. The author tentatively puts forward the suggestion that carotene plays some part in the production of the lower fatty acids in milk fat, since in his experiments the R.M. value of the fat increased with the carotene content of the fat and with the carotene intake of the animal. It need hardly be emphasized that such a suggestion founded on such slender evidence must be considered purely speculative. As the author, wisely, if perhaps a little unwillingly, points out, other dietary factors may also be concerned. Schloemer (199) has recorded the monthly average values for refractometer readings for almost nine thousand samples of butterfat collected between 1926 and 1939. A maximum was found in the summer months and a minimum in winter. Theophilus (200) records that he observed no significant change in the R.M. or in the iodine value when the external temperature was subject to sudden changes, as, for example, from 30 to 60° F.

The seasonal variations in the levels of fat and non-fatty solids of bulk milk as available in the Borough of Woolwich are shown in a report (201) in which the average values for data collected over a 5-year period are recorded. The fat content is consistently low in the months of April to June, and highest from October to December, the average values for the two periods being 3·43 and 3·79%. The fat content appears to be highest at the onset of the colder weather. In a thesis by Burr (202) analytical results obtained for milk samples from some twelve thousand farms distributed over thirteen districts of England and Wales during 1934–5 have been statistically analysed. No significant correlation was found between the non-fatty solids content of the milk and the height of the farm above sea level, the size of the herd, the type of soil, or the feeding of succulent foods in winter. Higher percentages of non-fatty solids were observed on farms where supplementary foods were fed in the summer, and where the conditions of the cows was good and the fat percentage in the milk was high.

The daily variation in milk yield and fat content has been studied by Erbacher (203) and also in great detail by Jarl (204). The latter author, who works at the Animal Breeding Station in Wiad, has collected the values for over six thousand individual samples obtained daily from twenty-five cows during 1929–30. These have been examined statistically and a number of correlations worked out for several relationships, such as those which might exist between milk yield and fat percentage and between daily milk yield and its variation. The paper contains so much statistical information that it is impossible to review it here.

The possible effect of sunshine, given in the form of ultra-violet light, on milk production has been studied by Krauss *et al.* (205). A group of Holstein cows received the equivalent of 100 hr. of midsummer sunshine in addition to the natural sunshine to which a control group was exposed. Under these conditions no difference in milk production was observed in the first year, nor was there any change in the second year when three times as much ultra-violet light was given. It may be mentioned here that ultra-violet ray treatment has been found beneficial in human lactation (206, 207).

One other publication which may be cited in this section deals with the use of cattle for draught purposes and the effect of this on their milk production. Tornede (208), investigating the problem for Red Mountain cattle in Germany, has proposed a work unit consisting of 8 hr. under normal working conditions. He found no reduction in milk yield or in fat percentage if the animals did less than 20 work units per lactation, but with 120 units there was a reduction in yield of some 30%.

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(*MS. received for publication* 13 *December* 1940)